VOL. XIV, No. 3 MAY 1961

UNIVERSITY OF HAWAII
LIBRARY
AUG 22 '61

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

MARTINUS NIJHOFF — PUBLISHER — THE HAGUE

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol

Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten

Issued under the auspices of the "Koninklijk Genootschap voor Landbouwwetenschap" (Royal Netherlands Society of Agricultural Science)

Executive Editors: D. I. Arnon, Berkeley; J. Baeyens, Louvain; F. C. Gerretsen, Groningen; G. W. Harmsen, Groningen; E. G. Mulder, Wageningen; F. J. Richards, London; A. C. Schuffelen, Wageningen; F. Steenbjerg, Copenhagen. **Secretary:** G. H. Arnold, Groningen.

Consulting Editors: Wm. A. Albrecht, Columbia, Mo.; C. Arnaudi, Milan; E. Gäumann, Zurich; L. Gisiger, Liebefeld-Berne; F. G. Gregory, London; A. J. de Groot, Groningen; W. R. C. Handley, Oxford; J. L. Harley, Oxford; J. den Holder, Ghent; V. J. Koningsberger, Utrecht; J. Lavollay, Paris; L. Leyton, Oxford; W. J. Lütjeharms, Bloemfontein; H. Lundegårdh, Uppsala; E. Melin, Uppsala; M. Ødelien, Ås; E. J. Petersen, Copenhagen; C. S. Piper, Adelaide; D. Snow, Reading; H. G. Thornton, Harpenden, Herts.; S. Tovborg Jensen, Copenhagen; A. I. Virtanen, Helsinki; S. A. Waksman, New Brunswick; F. W. Went, Pasadena; Joh^a. Westerdijk, Baarn; L. K. Wiersum, Groningen; J. G. Wood, Adelaide.

The Editors are - if necessary - advised by experts from the Staff of the Institute for Soil Fertility, Groningen

CONTENTS: TABLE DES MATIÈRES: INHALT:

A. D. Rovira and *J. R. Harris*, Plant root excretions in relation to the rhizosphere effect. V. The exudation of B-group vitamins 199

G. C. Papavizas and *C. B. Davey*, Extent and nature of the rhizosphere of lupinus. 215

E. A. McComb and *V. V. Rendig*, Effect of nutritional stress on plant composition. III. Characterization of the pectic substances in normal and sulfur-deficient alfalfa 237

Randolph T. Wedding and *M. Kay Black*, Uncoupling of phosphorylation in Chlorella by 2,4-dichlorophenoxyacetic acid 242

P. R. Hesse, The decomposition of organic matter in a mangrove swamp soil 249

F. Scheffer, B. Ulrich und *H. J. Kaufmann*, Über die Phosphationenaufnahme durch Pflanzen. I. Phosphationenaufnahme durch Hafer als Funktion der Phosphatkonzentrationen in Nährlösung und Pflanze. 264

J. R. Seeger, Le problème de l'excrétion d'azote par les légumineuses. Premières recherches en région équatoriale 277

Werner Rathje, Abhängigkeit der Löslichkeit der Phosphorsäure und des Kalis im Boden von der potentiellen Wasserstoffionenkonzentration 289

Printed in the Netherlands

Plant and Soil XIV, 199-296 The Hague May 1961

Plant and Soil XIV, no 3 May 1961

PLANT ROOT EXCRETIONS IN RELATION TO THE RHIZOSPHERE EFFECT

V. THE EXUDATION OF B-GROUP VITAMINS

by A. D. ROVIRA and J. R. HARRIS

C.S.I.R.O., Division of Soils, Adelaide, South Australia

INTRODUCTION

In spite of the considerable amount of evidence for the exudation of many organic substances from the roots of plants, there is relatively little information on growth factors present in root exudates. The only report of measured exudation of identified growth factors is that of West [18] who reported significant quantities of biotin and thiamine from flax roots grown aseptically in nutrient solution. The validity of these thiamine levels is now in doubt because the *Staphylococcus aureus* assay method of West and Wilson [19] also responds to the thiazole plus pyrimidine components of thiamine (Schopfer [13]). Bhuvaneswari and Sulochana [4] have reported the presence of substances having "thiamine-biotin replacement value" in the exudates from the roots of a number of plants but no distinction was made between these two vitamins. The exudation from tomato and pine roots of unidentified growth factors which promoted the growth of mycorrhizal fungi in the presence of salts, sugars and B-vitamins was reported by Melin [9]. Subsequently, Melin and Ramas Das [10] reported that this substance ("M" factor) was exuded by a range of plants.

The present investigation was undertaken with the aim of quantitatively assessing the amount of the various members of the B-group vitamins which are exuded by the roots of a number of plants species. The effects of different environmental conditions upon this exudation were also studied.

MATERIALS AND METHODS

(a) *Preparation of exudate*

(i) Plants. Ten plants were used, *viz*: – Tomato, (*Lycopersicon esculentum* Mill.) var. "South Australian Early Dwarf", field pea (*Pisum arvense* L.) var. "White Brunswick", subterranean clover (*Trifolium subterraneum* L.) var. "Bacchus Marsh", lucerne (*Medicago sativa* L.) var. "Hunter River", white clover (*Trifolium repens* L.) var. Ladino (U.S.A.), berseem clover (*Trifolium alexandrinum* L.), clustered clover (*Trifolium glomeratum*, L.), crimson clover (*Trifolium incarnatum* L.), strawberry clover (*Trifolium fragiferum* L.), phalaris (*Phalaris tuberosa* L.).

(ii) Seed sterilization and plant culture. The seeds were surface-sterilized by shaking with a 7% calcium hypochlorite filtrate for two hours for peas and one hour for the remaining seeds. Germination of the seeds was carried out on tapwater agar so that the medium could not supplement the vitamin content of the germinated seeds and influence the composition of the exudate. The plants were grown by the method described by Rovira [11] except that distilled water replaced the plant-nutrient solution in all experiments other than that in which the effect of plant nutrition on exudation was studied. In this case a 1-in-4 dilution of Hoagland and Arnon [7] solution was used. The use of distilled water or dilute plant-nutrient solution obviated the need to desalt the solution following concentration prior to assay. The solutions supporting the roots were changed at the intervals, as shown in Tables 1 to 9, and concentrated at 30°C in a rotary film evaporator (Craig *et al.*[5]). The final volume of the concentrate was adjusted to provide the equivalent of exudate from four root systems per millilitre.

The effect of light intensity upon plant growth and exudation was studied by shading plants with perforated metal cages (Rovira [11]). In this way incident daylight was reduced to 40% in the low light-intensity treatment. In experiments upon effects of temperature, limited control was achieved by growing plants in ventilated glass boxes 90 × 60 × 60 cm, fitted with supplementary heaters. The temperature ranges were:– "low" treatment, 16°C (Min.) to 30°C (Max.) and "high" treatment, 24°C (Min.) to 36°C (Max.). These correspond to the "low" and "medium" temperatures of previous experiments described by Rovira [11].

(b) *Bioassay of vitamins*

The vitamin content of root-exudate concentrates was determined by bioassay methods using a test organism with essential growth-factor requirement grown in medium free of that factor. Standard response curves were obtained by adding the factor in a range of specified concentrations; the growth response of the same organism to root exudate was then compared to the standard. The growth response of the test organism was measured both acidimetrically and turbidimetrically for bacteria, and gravimetrically for the fungus.

The assay media was reconstituted from Difco dehydrated culture media.

Stock cultures of Lactobacilli were carried in stabs of Micro Assay Culture Agar (B319, Difco Manual [6]); the inoculum was prepared from growth for 24 hours at 35°C in Micro Inoculum Broth (B320), centrifuged and resuspended in isotonic saline. Stock cultures of *Neurospora sitophila* were carried on slants of *Neurospora* culture agar (B321), and a spore suspension in isotonic saline from 48 hour culture used as inoculum.

Bioassays using bacteria were done in 20 × 125-mm Kimble screw-cap culture tubes containing 10-ml aliquots of media. Turbidimetric determinations were made on each tube immediately after inoculation and after incubation at 35°C for 16 to 20 hours by measuring the percentage transmission at wavelength 660 mμ using a Unicam SP400 D.C. absorptiometer with modified tube-holder. After 72 hours (except for thiamine) acidimetric determinations were made by titrating the contents of each tube with 0.1 *N* sodium hydroxide using bromothymol blue as indicator. Bioassay by *Neurospora* was carried out in 50-ml erlenmeyer flasks plugged with cotton and containing 10-ml aliquots of medium. After 5 days growth at 30°C, the flasks were steamed, mycelial mats harvested, squeezed dry between tissue, rolled into pellets and dried on a spotting tile at 100°C for two hours in a vacuum oven, and mycelium weighed to constant weight.

(i) Biotin. The test organism used was *Lactobacillus plantarum* (*syn. L. arabinosus* 17–5) ATCC 8014 in Biotin Assay Medium (B419, Difco Manual [6]) modified from Skeggs and Wright [14]. Standard biotin tubes were prepared from a solution of crystalline biotin (free acid) diluted to give a range of 0.0 to 1.0 μmg per 10-ml aliquot medium in 12 steps. A standard curve for each assay was prepared by plotting mean values of percentage transmission and mean values of titration readings expressed in ml 0.1 *N* sodium hydroxide for each step of the range. The most effective assay range lay between 0.025 and 0.50 μmg per tube. Biotin content of exudate was estimated by matching response at suitable dilution to the standard curve.

(ii) Pantothenic acid. The test organism used was *Lactobacillus plantarum* (*syn. L. arabinosus* 17–5) ATCC 8014 in Pantothenate Assay Medium (B323, Difco Manual [6]), modified from Skeggs and Wright [14]. Standard pantothenic acid tubes were prepared from calcium pantothenate dissolved in 0.02 *N* acetic acid—sodium acetate (A.O.A.C.[1]), and diluted to give a range of 0.0 to 0.10 μg per tube in steps of 0.01 μg. Standard curves were prepared for tubidimetric and acidimetric readings. The most effective assay range was within 0.02 to 0.09 μg pantothenic acid per tube.

(iii) Niacin. The test organised was *Lactobacillus plantarum* (*syn. L. arabinosus* 17–5) ATCC 8014 in Niacin Assay Medium (B322, Difco Manual [6]), the medium of Snell and Wright [16] as modified by Barton-Wright [2] and adopted by A.O.A.C.[1]. Standard niacin tubes were prepared from an aqueous solution of nicotinic acid diluted to give a range of 0.0 to 0.50 μg per tube in steps of 0.05 μg. Standard curves were prepared for turbidimetric and acidimetric readings. The most effective assay range lay between 0.05 and 0.40 μg per tube.

(iv) Riboflavin (vitamin B_2). The test organism was *Lactobacillus casei* e ATCC 7469 in Riboflavin Assay Medium (B325, Difco Manual [6]) modified

from Snell and Strong [15] by reduction of dextrose to 2%. Standard riboflavin tubes were prepared from riboflavin dissolved in 0.02 *N* acetic acid and diluted with water to give a range of 0 to 250 μmg per tube in steps of 25 μmg. Standard curves were prepared for turbidimetric and acidimetric readings. The most effective assay range lay between 25 and 200 μmg per tube.

(v) Thiamine (vitamin B_1). The test organism was *Lactobacillus fermentum* 36 ATCC 9338 in Thiamine Assay Medium (B326 Difco Manual,[6]) of Sarett and Cheldelin [12]. Standard tubes were prepared from an aqueous solution of thiamine hydrochloride diluted to give a range of 0.0 to 0.05 μg per tube in steps of 0.005 μg. A standard curve for each assay was prepared from turbidimetric measurements after 16 to 18 hours growth at 35°C; acidimetric determinations cannot be used. The most effective assay range lay between 0.005 and 0.03 μg per tube.

(vi) Pyridoxine (vitamin B_6). The test organism was *Neurospora sitophila* 299 ATCC 9276 in Pyridoxine Assay Medium (Difco Manual, B324 [6]), the medium of Stokes *et al.*[17] as modified by Barton-Wright [3]. Standard flasks were prepared from an aqueous solution of pyridoxine hydrochloride to give a range of 0.0 to 1.0 μg per flask in steps of 0.1 μg. A standard curve was prepared by plotting mean dry weights of mycelium for each step of the range. The most effective assay range lay between 0.1 and 0.8 μg per flask.

RESULTS

(*a*) *Vitamin content of exudate*

The assessment of the role of accessory growth factors in influencing growth of micro-organisms in the rhizosphere, was made in relation to magnitude of response by test organisms rather than in absolute terms. On this basis, both the quantity and the numbers of vitamins appearing in the exudate were low. The results summarised in Table 1 show that the most active substance was biotin, although the absolute amount exuded was very small indeed; this was exuded in largest amounts by field pea, moderate amounts by lucerne, small amounts by white clover and tomato, and less by the grass *Phalaris tuberosa*. Each plant species tested showed amounts in the exudate sufficient to give significant growth responses by micro-organisms unable to synthesise biotin. Pantothenate was next in order of activity, but only in field pea, lucerne, and tomato were the amounts of a significant order. Very little niacin was found in the concentrate and the amount was significant only for field pea. Traces of riboflavin and thiamine were found in field-pea exudate, but for the others the bioassay was negative. Pyridoxine was absent at assayable levels in all concentrates.

TABLE 1

B-group vitamins in root exudates (concentrated to 4 plants per ml) from 5 plant species at two harvest times

Exudate	Vitamin and effective assay range					
Age in weeks	Biotin (0.025–0.50) μmg/ml	Thiamine (0.005–0.40) μg/ml	Riboflavin (0.025–0.20) μg/ml	Niacin (0.05–0.40) μg/ml	Panto-thenate (0.01–0.09) μg/ml	Pyrid-oxine (0.10–0.90) μg/ml
Lucerne						
0–1	0.75	Trace (?)	—	⩽ 0.1	0.045	—
1–2	0.65	—	—	0.06	0.048	—
White clover						
0–1	0.125	—	—	—	< 0.010	—
1–2	0.37	—	—	0.01	0.010	—
Field pea						
0–1	10	Trace (?)	—	0.4	0.100	—
1–2	16	< 0.0075	Trace (?)	1.6	0.320	—
Tomato						
0–1	0.25	—	—	⩽ 0.1	0.055	—
1–2	0.225	—	—	0.03	0.024	—
Phalaris						
0–1	0.10	—	—	⩽ 0.1	⩽ 0.010	—
1–2	0.125	—	—	0.02	0.017	—

Table 1 may be summarised by saying that other than biotin, and to a lesser extent pantothenate and niacin, the vitamin-B complex was not found to be present in appreciable quantities in root exudate harvested under the conditions of the experiment.

(b) Plant species

The data in Table 1 also show that pronounced differences occur from plant to plant. Field pea produced by far the greatest amounts of each of the B-group vitamins, and was the only plant species to show traces of thiamine and riboflavin, although devoid of pyridoxine. It was the largest plant used in the test series and made appreciable growth during the experiment (Table 2). The other leguminous plants, lucerne and white clover, also gave moderate yields, but it is dangerous to draw generalisations at the family level when tomato yielded as much biotin and more pantothenate and niacin than did white clover. Of the species under test, the grass *Phalaris tuberosa* gave the lowest yields and was the smallest plant in the test series (Table 2). Whilst it is likely that patterns exist in the way the B-group vitamins are exuded, all that can be said is that

TABLE 2

Fresh weights of five plant species at two harvest times (mg per plant)

Plant species	Age 7 days		Age 14 days	
	Tops	Roots	Tops	Roots
Lucerne	34.5	5.3	20.6	15.0
White clover	6.1	2.7	5.9	4.2
Field pea	626	230	764	396
Tomato	25.0	8.9	33.5	11.6
Phalaris	8.9	3.0	11.4	8.9

individual plant species show differences which could not easily be correlated with size, *e.g.* lucerne and tomato of comparable size (Table 2) gave large differences in biotin exuded (Table 1).

(*c*) *Age of plant*

The data of Table 1 show that the exudation of vitamins during the first week after germination differs very little from that of the second week on a per plant basis. The harvested fresh weights of plants show considerable differences from the first to the second week. This is particularly reflected in the growth of roots (Table 2), and therefore it would seem that exudation from the seedlings diminished with time on a unit weight of tissue basis.

(*d*) *Light intensity*

It can be seen in Table 3 that a reduction in light intensity did not produce large or clear-cut influences on total vitamin exudation of several plant species; trends found for one vitamin did not necessarily hold for others and different reactions were given by different plant species.

Tomato showed little effect of reduced light intensity on either vitamin content (Table 3) or plant weight (Table 4). There were no appreciable differences in the amounts of biotin; traces of niacin were found only at the lower light intensity. Subterranean clover exuded more biotin and a trace of thiamine at high light intensities; there were no differences in fresh weights of plants. Lucerne exuded more biotin and traces of riboflavin and pantothenate at low light intensities; there were no differences in fresh weights of plants.

Field pea showed better growth response to high light intensities

TABLE 3

Influence of age of plant and light intensity upon exudation of B-group vitamins by four plant species

Exudate		Vitamin and effective assay range					
Age in weeks	Light intensity, %	Biotin (0.025–0.50) μmg/ml	Thiamine (0.005–0.40) μg/ml	Riboflavin (0.025–0.20) μg/ml	Niacin (0.05–0.40) μg/ml	Panto-thenate (0.01–0.09) μg/ml	Pyrid-oxine (0.1–0.9) μg/ml
Tomato							
0–1	100	0.019	—	—	—	—	—
	40	0.019	—	—	0.010	—	—
1–2	100	0.012	—	—	—	—	—
	40	0.015	—	—	0.010	—	—
Subterranean clover							
0–1	100	0.045	0.019	—	0.010	—	—
	40	0.025	—	—	0.015	0.004	—
1–2	100	0.045	0.010	—	—	0.002	—
	40	0.014	—	—	—	—	—
Lucerne							
0–1	100	0.014	—	—	0.010	—	—
	40	0.170	—	< 0.005	0.010	—	—
1–2	100	0.025	—	—	—	—	—
	40	0.035	—	—	—	0.001	—
Field pea							
0–1	100	0.20	—	< 0.005	0.025	0.001	—
	40	0.36	—	—	0.025	0.012	—
1–2	100	1.42	—	—	0.037	—	—
	40	0.40	—	—	0.140	0.0025	—

as the harvest weights of tops and roots show in Table 4, but this cannot be correlated with the trends in exudation of vitamins shown in Table 3 where biotin was increased and a trace of riboflavin found at high light intensities but more niacin and pantothenate were exuded at low light intensities.

(*e*) *Temperature*

Tomato showed greater exudation of vitamins at lower temperatures. This is shown in the data of Table 5 for biotin and for the trace

TABLE 4

Fresh weights of four plant species under two light intensities at two harvest times (mg per plant)

Plant species and percentage light intensity		Age 7 days		Age 14 days	
		Tops	Roots	Tops	Roots
Tomato	100%	18.7	7.0	24.5	10.0
	40%	16.8	7.3	25.4	8.8
Subterranean clover	100%	74	19.0	78	25
	40%	78	18.5	78	18
Lucerne	100%	12.7	8.3	17.8	7.5
	40%	15.6	8.1	17.5	8.1
Field pea	100%	540	223	755	421
	40%	587	193	645	257

TABLE 5

Influence of temperature and age of plant upon exudation of B-group vitamins by tomato and subterranean clover seedlings

Exudate		Vitamin and effective assay range					
Age in weeks	Growth temperature *	Biotin (0.025–0.50) µmg/ml	Thiamine (0.005–0.40) µg/ml	Riboflavin (0.025–0.20) µg/ml	Niacin (0.05–0.40) µg/ml	Pantothenate (0.01–0.09) µg/ml	Pyridoxine (0.1–0.9) µg/ml
Tomato							
0–1	Low	0.033	0.010	< 0.005	—	0.001	—
	High	0.025	—	—	—	—	—
1–2	Low	0.025	—	—	—	—	—
	High	0.010	—	—	—	—	—
Subterranean clover							
0–1	Low	0.118	0.010	0.010	0.015	0.010	—
	High	0.089	—	—	0.016	0.006	—
1–2	Low	0.083	0.010	—	0.015	0.013	—
	High	0.250	0.009	< 0.005	0.025	0.011	—

* Low temperature = 16°C (Min.) to 30°C (Max.).
High temperature = 24°C (Min.) to 36°C (Max.).

TABLE 6

Fresh weights of seedlings of tomato and subterranean clover grown under two temperature regimes and at two harvest times (mg. plant)

Plant species and growth temperature	Age 7 days		Age 14 days	
	Tops	Roots	Tops	Roots
Tomato				
Low	23.6	8.7	23.1	10.5
High	24.3	12.9	26.3	7.3
Subterranean clover				
Low	83.5	24.1	67.2	25.7
High	[illegible]	23.5	52.9	22.9

quantities of thiamine, riboflavin and pantothenate; no niacin was found. Subterranean clover on the other hand exuded more biotin and more niacin at higher temperatures, but at lower temperatures there was more pantothenate, more riboflavin and traces of thiamine The fresh-weight data for the plants (Table 6) shows that there was little appreciable influence of temperature except tomato tended to show more rapid growth at higher temperature.

(*f*) *Sterility*

The results of the previous experiments had been obtained using surface-sterilized seeds grown in test tubes under ordinary aseptic precautions. In order to assess the influence of a normal microflora derived from the seed-coat, about one hundred seeds were washed in 10 ml sterile distilled water to provide an inoculum from which 0.1 ml of suspension was added to each test tube at time of transplanting. The sterile set received an aliquot of the same seed-washings sterilized by autoclaving.

From Table 7 it can be seen that tomato and subterranean clover each showed maximal vitamin contents in exudates from the sterilized series, although fresh weights of plants did not indicate effects upon plant growth. Under non-sterile conditions, the reduction in biotin was dramatic. In the case of subterranean clover where there was appreciable exudation, the amount was reduced to one third; tomato with very little biotin exuded showed complete loss due to micro-organisms. Similar trends were shown for pantothenate.

TABLE 7

Influence of rhizosphere micro-organisms and age of plant upon exudation of vitamins and harvest weights of seedlings of tomato and subterranean clover

Age in weeks	Presence of rhizosphere micro-organisms	Fresh weight of plants		Vitamin and effective assay range		
		Tops mg/plant	Roots mg/plant	Biotin (0.025–0.5) μmg/ml	Niacin (0.05–0.4) μg/ml	Panto-thenate (0.01–0.09) μg/ml
Subterranean clover						
0–1	Sterile	76.0	19.6	0.045	< 0.1	0.013
	Non-sterile	84.5	21.0	0.012	< 0.1	—
1–2	Sterile	75.0	19.0	0.044	—	< 0.005
	Non-sterile	76.0	23.5	0.015	—	—
Tomato						
0–1	Sterile	19.7	8.6	0.015	—	< 0.005
	Non-sterile	18.0	7.5	—	< 0.01	—
1–2	Sterile	24.5	12.0	0.012	< 0.1	< 0.005
	Non-sterile	21.5	12.6	—	< 0.1	—

TABLE 8

Influence of mineral nutrition and age of plant upon exudation of vitamins and harvest weights of seedlings of tomato and subterranean clover

Age in weeks	Growth solution	Fresh weights of plants		Vitamin and effective assay range		
		Tops mg/plant	Roots mg/plant	Biotin (0.025–0.5) μmg/ml	Niacin (0.05–0.4) μg/ml	Panto-thenate (0.01–0.09) μg/ml
Subterranean clover						
0–1	Nutrient solution	109	21.3	0.04	0.1	< 0.005
	Distilled water	95.9	19.3	—	< 0.1	0.01
1–2	Nutrient solution	134	30.0	0.04	0.2	< 0.005
	Distilled water	90.7	22.6	—	< 0.1	0.0075
Tomato						
0–1	Nutrient solution	43.8	10.7	—	< 0.1	< 0.005
	Distilled water	19.2	2.3	—	< 0.1	< 0.005
1–2	Nutrient solution	69.1	14.2	—	0.15	< 0.005
	Distilled water	23.7	10.8	—	< 0.1	< 0.005

(g) Mineral nutrition

All previous experiments had been conducted with distilled water as the liquid in the test tubes in which plants were grown. In order to ascertain whether mineral nutrition of the plant influenced the exudation of vitamins, the mineral solution of Hoagland and Arnon [7] was substituted as a plant nutrient solution, but to reduce salting out during concentration, it was used at one-quarter strength.

The data in Table 8 shows that improved supply of plant nutrients increased the exudation of vitamins as well as plant growth. In this experiment tomato was a poor source of any vitamin except niacin; this may have been due to possible contamination of the plants during this experiment. Subterranean-clover exudates contained more biotin and more niacin in the nutrient solution series, but less pantothenate. The only explanation for this latter observation is that the glasshouse programme was carried out during the winter months giving the low-temperature effect coupled with less total growth in distilled water; under these conditions the exudation of pantothenate would be increased.

(h) Seed size and clover species

Previous experiments had shown that the exudation of vitamins was greatest for seedling of large size, *viz* field pea, and least for that of small size, *viz Phalaris tuberosa*, subterranean clover exuded most vitamins under conditions of maximal plant growth *i.e.* for plants grown in plant-nutrient solution. In order to resolve the importance of seed size and plant size upon exudation, and in order to show relationships between species within the same genus, an experiment was conducted with five species of clover, *viz Trifolium subterraneum* L. (subterranean clover), *T. alexandrinum* L. (berseem), *T. fragiferum* L. (strawberry clover), *T. glomeratum* L. (clustered clover) and *T. incarnatum* L. (crimson clover). Within this group, the subterranean clover seed was graded into two sizes by passing through screens to give samples with diameter less than 1.9 mm (small seed) and greater than 2.6 mm (large seed). After germination on agar, the seedlings were transferred to tubes containing one-quarter strength plant-nutrient solution, and the exudate harvested after ten and twenty days.

Subterranean clover from seed of different size gave rise to plants

TABLE 9

Influence of seed size and plant size upon exudation by five species of clover

Clover	Age at harvest, days	Seed size	Fresh weights of plants		Vitamin and effective assay range		
			Tops	Roots	Biotin	Niacin	Pantothenate
			mg/plant	mg/plant	(0.025–0.5) μmg/ml	(0.05–0.4) μg/ml	(0.01–0.09) μg/ml
Subterranean clover	0–10	small	85	17.4	0.42	< 0.1	0.030
		large	153	26.0	0.30	< 0.1	< 0.005
Subterranean clover	11–20	small	99	19.9	0.17	< 0.1	0.011
		large	175	32.4	0.26	0.125	0.018
Strawberry clover	0–10		19.0	5.1	0.15	< 0.1	0.047
	11–20		50.8	7.1	0.12	< 0.1	0.023
Berseem	0–10		47.0	12.9	0.20	< 0.1	0.020
	11–20		50.2	17.3	0.26	0.14	0.019
Crimson clover	0–10		53.0	9.2	0.12	< 0.1	0.012
	11–20		50.2	17.3	0.09	< 0.1	0.008
Clustered clover	0–10		11.4	3.5	0.05	< 0.1	0.006
	11–20		51.0	7.1	0.04	< 0.1	0.006

of different fresh weights, the large seeds giving seedlings of greater weights of both tops and roots sustained over the period of the experiment (Table 9). Total biotin exudation was the same for plants from both small and large seeds although small seed plants produced more biotin in the first ten days than in the second, and produced more pantothenate than did the large. Niacin exudation was small, but more was produced from large seed plants.

The other four clovers produced seedlings of comparable top weights after twenty days, but root growth by clustered clover and strawberry clover was much less than that of berseem and crimson clover (Table 9). Despite the close botanical and size relationships of these plants, vitamin exudation showed variations quite unrelated to seed size or plant size.

(i) Seasonal trends in vitamin exudation

The much lower amounts of vitamins exuded by tomato and subterranean clover in some experiments (Tables 3, 7, and 8) than in others (Tables 1 and 9) may have been due to the former experiments being conducted during winter whereas the experiments in which high yields were recorded were carried out in summer and early autumn. However, further experiments with controlled-

environment growth cabinets would be necessary to establish the importance of day length, temperature and light intensity in this apparent seasonal trend in the exudation of vitamins by roots.

DISCUSSION

Of the numbers of the B-vitamin group for which assay were made, biotin was the only vitamin which consistently appeared in the exudate in significant quantities. Pantothenate and niacin were often present but were generally in quite low amounts, riboflavin and thiamine occurred infrequently and then only in trace amounts, while pyridoxine could not be detected.

According to Schopfer [13], plant roots synthesize biotin, but not thiamine, pantothenate, pyridoxine or niacin. It is not surprising then that biotin was consistently present in amounts significant to influence microbiological activity. These amounts are of the micro-milligram order due to the high biological activity of biotin. In view of Schopfer's report it is surprising that the less sensitive bioassays for microgram amounts of pantothenate and niacin so often gave positive responses and for amounts which in absolute terms but not in terms of microbiological activity, exceeded that of biotin.

The results have indicated that different plant species exude different amounts of the vitamins but no distinctive general patterns emerged. The variations in biotin and pantothenate in the exudates from the various clover species illustrate the inconsistency; *e.g.* from biotin exudation, subterranean = berseem > crimson = strawberry > cluster, while for pantothenate, strawberry > subterranean = berseem = crimson = cluster. This species difference was not related to seed size or plant size but appears to be a characteristic of plant species. In the case of extremes in seed size *e.g.* pea and phalaris it is not surprising that the large-seeded plant which produced a large root system released greater amounts of growth factors. During the initial seedling stages of growth at which these experiments were conducted there appeared to be little, if any, effect of age on vitamin exudation.

A study of the influence of various environmental conditions failed to reveal consistent effects of light intensity or temperature except for slight increases in pantothenate release at low light intensity and low temperature. This finding is in contrast to that in which it was

shown that amino acid exudation was markedly increased at high light intensity and high temperature (Rovira [11]).

Improved nutrition of tomato and subterranean clover plants were accompanied by increased exudation of biotin and niacin, but not of pantothenate. This may be the direct effect of improved plant growth, *e.g.* with plant-nutrient solution compared to distilled water, and in such cases the exudation of pantothenate was generally found to increase under less favourable growing conditions.

The marked reduction of biotin and pantothenate in the root exudate of subterranean clover and tomato when micro-organisms are present in the root environment illustrates the importance of maintaining complete asepsis in any critical root-exudate studies. The organisms introduced into the rhizosphere from the seed inoculum were capable of utilizing biotin and, to a lesser degree, pantothenate, and so exhausted it. There appeared to be no synthesis and release into the medium of these or others of the B-vitamins by the microflora, but possibly the duration of the experiment was insufficient to detect release of microbially synthesised vitamins. Older plants, supporting higher rhizosphere populations, may have an appreciable turn-over of growth factors. In the soil where intense microbial competition occurs with consequent rapid shifts in populations, synthesis and release of growth factors could be quite important. Lochhead [8] found that about one half of the soil isolates and somewhat more than half of the rhizosphere isolates synthesized one or more of the following growth factors, thiamine, biotin, riboflavin, vitamin B_{12} and "terregens" factor. In the rhizosphere this may mean that micro-organisms themselves supply the bulk of the growth factors produced thus supplementing the often meagre amounts released by the plant roots.

SUMMARY

The root exudates from seedlings of ten plant species grown under conditions of controlled environment and nutrition were biassayed for six vitamins of the B-group. Biotin was consistently present in the exudates in amounts sufficient to influence the growth of rhizosphere micro-organisms. Pantothenate and niacin were generally present, but usually at low levels unlikely to influence the microflora; riboflavin and thiamine were occasionally found in traces; pyridoxine was not detected in any root exudate.

The vitamin content of the exudate varied with plant species. Field pea

released large quantities of biotin, pantothenate, and niacin, but other plants including legumes, produced exudates medium to low in vitamin content and varying in relative amounts of each. Subterranean clover produced moderate amounts of vitamins, and from seed samples of graded size exuded vitamins in quantities unrelated to seed size. A comparison of five species of clover showed distinct differences in patterns of exudation in closely related plant species.

Raising temperature and reducing light intensity by shading, produced only small effects upon vitamin exudation. Improved nutrient status produced marked increases in plant growth, but only small increases in amount of vitamin exuded, with pantothenate an exception tending to be released in greater amounts under unfavourable growing conditions. The presence of a root microflora caused sharp reduction in vitamin concentration of the culture solution.

ACKNOWLEDGEMENT

We wish to acknowledge the valuable technical assistance of Miss. M. Ozolins and Miss. G. Banos.

Received May 31, 1960

REFERENCES

1 A.O.A.C., Official Methods of Analysis of the Association of Official Agricultural Chemists. Eighth edition. Association of Official Agricultural Chemists, Washington D.C., pp. 1008 (1955).
2 Barton-Wright, E. C., The microbiological assay of nicotinic acid in cereals and other products. Biochem. J. **38**, 314–319 (1944).
3 Barton-Wright, E. C., The theory and practice of the microbiological assay of the vitamin-B complex; together with the assay of selected amino acids and potassium. Analyst **70**, 283–295 (1945).
4 Bhuvaneswari, K. and Sulochana, C. B., Assay of root exudates. Current Sci. **24**, 376–377 (1955).
5 Craig, L. C., Gregory, J. D., and Haumann, W., Versatile laboratory concentration device. Anal. Chem. **22**, 1462 (1950).
6 Difco Manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. Ninth Edition. Difco Laboratories Incorporated, Detroit, Michigan, pp. 350 (1953).
7 Hoagland, D. R. and Arnon, D. I., The water culture method of growing plants without soil. Univ. Calif. Agr. Expt. Sta. Circ. **347** (1938).
8 Lochhead, A. G., Qualitative studies of soil micro-organisms: XV. Capability of the predominant bacterial flora for synthesis of various growth factors. Soil Sci. **84**, 395 (1957).
9 Melin, E., Growth factor requirements of mycorrhizal fungi of forest trees. Svensk Botan. Tidskr. **48**, 86 (1954).
10 Melin, E., and Ramas Das, V.S., Influence of root metabolites on the growth of tree mycorrhizal fungi. Physiol. Plantarum **7**, 851 (1954).

11 Rovira, A. D., Root excretions in relation to rhizosphere effect. IV. Influence of plant species, age of plant, light, temperature and calcium nutrition on exudation. Plant and Soil **11**, 53–64 (1959).
12 Sarett, H. P. and Cheldelin, V. H., The use of *Lactobacillus fermentum* 36 for thiamine assay. J. Biol. Chem. **155**, 153–160 (1944).
13 Schopfer, W. H., Plants and Vitamins. Publ. Chromica Botanica Company, Waltham, Mass. (1949).
14 Skeggs, H. R. and Wright, L. D., The use of *Lactobacillus crabinosus* in the microbiological determination of pantothenic acid. J. Biol. Chem. **156**, 21–26 (1944).
15 Snell, E. E. and Strong, F. M., A microbiological assay for riboflavin. Ind. Eng. Chem. Anal. Ed. **11**, 346–350 (1939).
16 Snell, E. E. and Wright, L. D., A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**, 675–686 (1941).
17 Stokes, J. L., Larson, A., Woodward, C. A., and Foster, J. W., A *Neurospora* assay for pyridoxine. J. Biol. Chem. **150**, 17–24 (1943).
18 West, P. M., Excretion of biotin and thiamine by roots of higher plants. Nature **144**, 1050 (1939).
19 West, P. M. and Wilson, P. W., Biological determination of vitamin B_1 (thiamin) in *Rhizobium trifolii*. Science **88**, 334–5 (1938).

Plant and Soil XIV, no. 3 May 1961

EXTENT AND NATURE OF THE RHIZOSPHERE OF LUPINUS

by G. C. PAPAVIZAS and C. B. DAVEY

Crops Research Division, Agricultural Resarch Service,
U.S. Department of Agriculture, Beltsville, Maryland

INTRODUCTION

Since 1904 when Hiltner [8] introduced the term "rhizosphere" to denote that portion of the soil near and under the influence of plant roots, many investigators emphasized the intensive microbiological activity characteristic of this habitat. Comprehensive accounts (Clark [6], Ishizawa *et al.* [9], Katznelson *et al.* [11], Lochhead [13], Starkey [20]) published during the last 12 years, summarize most of our present knowledge on the rhizosphere and details need not be repeated here.

Although it seems plausible that proximity to plant roots influences microbial activity and that the effect of the root diminishes with distance from the root surface, only limited studies dealing with the extent of the root effect were reported. Starkey [19], during his pioneer work on rhizosphere micro-organisms of several agricultural plants, found consistently greater microbial numbers in soil samples collected from the immediate vicinity of roots of the test plants than in samples collected 12 to 30 cm from the roots. Thom and Humfeld [21] also observed higher numbers of micro-organisms in soil in proximity to roots of certain plants than in soil away from the roots. They observed that the numbers of micro-organisms associated with the roots themselves exceeded those in "soil adjacent to roots" as well as those in soil far away from the roots.

Clark [6] introduced the term "rhizoplane" to denote the external plant-root surface and closely adhered particles of soil and debris

and kept the term rhizosphere to describe the soil region adjacent to plant roots. The distribution of micro-organisms within the rhizoplane of certain plants was studied in considerable detail by various workers (Harley and Waid [7], Ishizawa *et al.* [9], Peterson [15]) who employed serial washings of roots or fractionation of samples by shaking the roots in air and then immersing them in water several times. From the rhizoplane outward to a distance of 10 cm the distribution of micro-organisms has not been studied extensively. Rovira [18] studied the extent of the root effect at various distances from the roots of certain plants and concluded that the rhizosphere effect was not evident more than 5 mm from the roots. However, he did not state his method of sampling.

It is now established that the microbial population of the rhizosphere differs both quantitatively and qualitatively from that of the non-rhizosphere soil (Katznelson *et al.* [11], Starkey [20]). The amount of information on bacteria is considerably greater than that on actinomycetes or fungi. However, several aspects of soil and rhizosphere fungi have received some attention. As early as 1932 Thom and Humfeld [21] reported that fungi were 40 to 70 times more numerous near corn roots than in soil distant from the roots when corn was grown in acid soil, but in alkaline soil the difference was only 2- to 5-fold. Although Timonin [22] failed to find significant differences among fungal populations in the rhizospheres of various plants or between the rhizosphere and the soil distant from it, he later [24] reported that the roots of varieties of flax susceptible to Fusarium wilt preferentially encouraged certain genera of fungi. Quantitative and qualitative differences also were found between fungi of the rhizosphere and those of the soil at a distance from the roots of other plants (Katznelson and Richardson [12], Peterson [15]). The fungal flora of the rhizosphere changed with plant age and soil type (Adati [1], Agnihothrudu [2], Katznelson and Richardson [12], Peterson [15], Starkey [19]), with soil treatment (Katznelson and Richardson [12], West and Hildebrand [27]), with foliar applications of chemicals (Ramachandra-Reddy [17]), and with mechanical injuries inflicted either above or below the crown (Clark [5]).

In the present study attempts were made (a) to study the distribution of microflora, with particular emphasis on fungi, from the rhizoplane of *Lupinus angustifolius* L. (blue lupine) outwards

and (b) to obtain information on the effect of various decomposing plant materials and nitrogen on the quantitative and qualitative distribution of fungi in the rhizoplane and at various distances from root surfaces of healthy blue lupine seedlings.

MATERIALS AND METHODS

Elsinboro sandy loam or a loamy sand greenhouse soil was passed through a 5-mm sieve, mixed thoroughly, weighed, and placed in tared No. 10 cans provided with holes for aeration and drainage. In experiments involving amended soils dry mature plant materials were added to the soils at the rate of 1 per cent of the oven-dry weight of the soil and the plant material. The plant materials used were barley straw, corn stover, and soybean meal. Except when otherwise noted, barley straw and corn stover were ground in a Wiley mill to pass through a 1-mm screen before use. Portions of the plant materials received nitrogen from readily available NH_4NO_3 at a concentration equivalent to 2 per cent by weight of the plant material added, or 200 mg N/kg soil. All plant materials were autoclaved at 1 pound pressure for 1 hour. After the unamended soils and soil–plant material mixtures were weighed (2.5 kg soil) and placed in the containers they were brought to 50 per cent of the moisture-holding capacity of the soil and maintained at that moisture content by periodic additions of water. All treatments were set up in quadruplicate in the greenhouse and a randomized-block design was used throughout. The air temperature in the greenhouse ranged from 23 to 27°C. Since the experiments were carried out from October through May light conditions varied somewhat from one experiment to another.

In all experiments blue lupine seeds were surface-sterilized for 1 minute with 1 per cent NaClO solution and rinsed with sterile distilled water, and one seed was planted in the center of each can. In experiments employing unamended soil throughout, the seeds were planted immediately after the soil had been placed in the containers. In experiments employing both amended and unamended soils the lupine seeds were planted 4 weeks after plant materials were added to the soil.

A soil microsampler was built for taking serial samples outward from the rhizoplane of the lupine plants in 3-mm increments. The microsampler (Plate 1) was fabricated in 2 parts: the sample collector and the sample ejector. The sample collector consisted of 7 thin-walled steel tubes, 50-mm long with an inside diameter of 3 mm, soldered adjacent to each other in a plane with an overall width of 22 mm. The sample ejector was made by setting 7 steel rods, 51 mm long with an outside diameter of 2.75 mm, in a $6 \times 6 \times 40$-mm brass bar in positions to match the tubes of the sample collector.

The method of sampling consisted of removing aseptically the top 2 cm of soil and then inserting the sterile sample collector vertically into the soil immediately adjacent and parallel to the primary root of the lupine seedling.

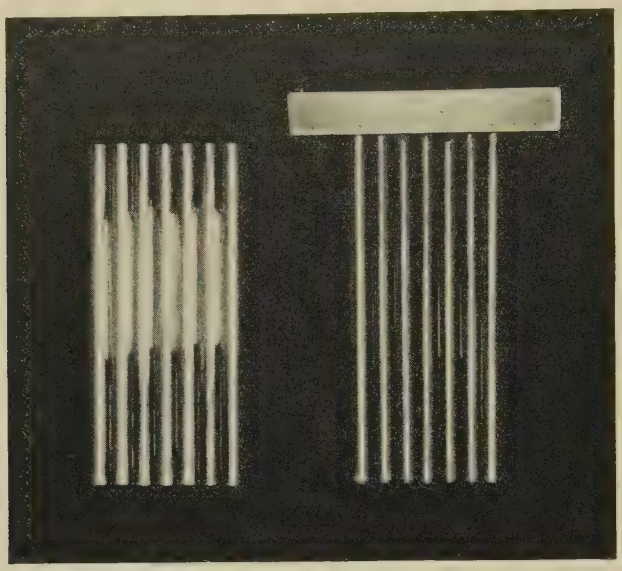

Plate 1. The microsampler (9/10 of the actual size). *Left*, sample collector. *Right*, sample ejector.

The sample collector was removed from the soil and the sterile sample ejector used to deliver soil cores of desired length into sterile water blanks. Each rod of the ejector was used with its corresponding tube of the collector to avoid cross contamination. Sample weights were fairly well controlled by collecting 1 mm of soil-core length for each 10 mg of soil sample desired. However, actual oven-dry sample weights were determined and data corrected accordingly.

Blue lupine was selected for this study because during the first 3 weeks of its growth it develops a straight primary root and 3 sets of secondary roots whose direction and orientation in soil is predictable. Two sets of the secondary roots grow from the primary root following the same direction pointed out by the 2 cotyledons which rise from the soil with the primary leaves, whereas the third set of secondary roots develops between the other two (Fig. 1). An arc of about 180° is free from secondary roots. The microsampler was inserted adjacent and parallel to the primary root in the middle of the area free from secondary roots. Thus serial microsamples were simultaneously collected from the root surface outward in 3-mm increments. The first core, therefore, was a portion of soil parallel to the root surface 0 to 3 mm from the root. It was not feasible to use the microsampler with plants which had fibrous root systems or with plants whose root direction in soil was unpredictable.

When the microsampler was not used, the method of taking rhizoplane samples consisted of removing superfluous soil from the plant roots but

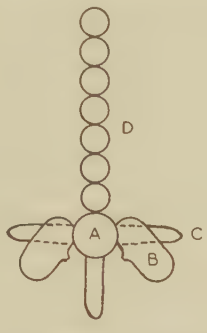

Fig. 1. Diagram illustrating the relation between a lupine seedling and the microsampler (top view):
A, stem or primary root; *B*, cotyledon; *C*, secondary root; *D*, microsampler.

retaining soil which adhered to them (Katznelson *et al.*[11]). The results of rhizoplane counts were expressed as numbers of organisms per gram of oven-dry gross sample including roots and adhered soil (Clark [6]). Preliminary experiments indicated that the dry weight of the young lupine roots comprised about 6 to 10 per cent of the total dry weight of the rhizoplane samples.

All dilutions in the original tared flasks were made 1 : 100 and were shaken on a rotary shaker for 30 minutes. Subsequent dilutions were shaken by hand for 1 minute. After the required dilutions had been prepared, the original flasks were dried to constant weight in an electrical oven at 105°C. The weights of oven-dry samples were used to calculate dilution coefficients. The dilutions of rhizosphere and non-rhizosphere soil (control) obtained with the microsampler were made on a 100-mg basis. All other dilutions of non-rhizosphere soil were made on a 5.0-g basis. In all samples 8 petri plates were used for each replication.

Soil, rhizosphere, and rhizoplane bacteria and streptomycetes were counted after 10 days of incubation at 30°C on dilution plates of egg albumin agar (Waksman [25]) supplemented with 50 ppm of cycloheximide to restrict fungal growth. Fungi from the same habitats were counted after 2, 3, and 4 days of incubation at 25°C on dilution plates of V-8 juice-dextrose-yeast extract agar (VDYA) supplemented with antimicrobial agents (Papavizas and Davey [14]).

Qualitative differences between fungi in the rhizoplane and fungi in soil at various distances from the root surfaces were determined by identification of the fungi on the original dilution plates, or when necessary, by transferring to special identification media.

To determine the effect of plant materials on root-surface fungi the roots were removed from the original dilution flasks, placed in sterile flasks, and washed with running tap water for 15 minutes. The washed roots were then transferred to clean sterile flasks, subjected to 5 serial washings

according to the method described by Harley and Waid [7], cut into 5 to 6 mm lengths, and plated on acidified potato-dextrose agar, corn-meal agar, VDYA, and water agar. Aureomycin hydrochloride, neomycin sulfate, and streptomycin sulfate (at 50 ppm each) were added to corn meal agar, VDYA, and water agar to prevent bacterial growth. From each root 25 pieces were plated on each of the 4 media. The plates were incubated at 25°C for 2 weeks.

EXPERIMENTAL RESULTS

Extent of rhizosphere of blue lupine in unamended soil

Micro-organisms in the rhizoplane (0 distance) of healthy blue lupine seedlings and in soil cores removed with the microsampler from various distances from seedling root surfaces were determined 18 days after planting. The experiment with Elsinboro sandy loam was carried out 3 times and that with loamy sand greenhouse soil twice. Representative data from one experiment are shown in Table 1. Results from the other experiments were comparable.

TABLE 1

Micro-organisms (thousands per g of oven-dry soil) at various distances from the root surface of lupine seedlings grown in unamended Elsinboro sandy loam

Distance from root surface (mm)	Bacteria	Streptomycetes	Fungi
0 *	159,000	46,700	355
0–3	49,700	15,500	176
3–6	38,000	11,400	170
9–12	37,400	11,800	130
15–18	34,170	10,100	117
80 **	27,300	9,100	91
R/S ratio †	5.8	5.1	3.9

* Rhizoplane.

** Non-rhizosphere soil (control).

† $\text{R/S ratio} = \dfrac{\text{No. of micro-organisms 0 mm from root surface}}{\text{No. of micro-organisms 80 mm from root surface}}$.

In unamended Elsinboro sandy loam the highest numbers of bacteria, streptomycetes, and fungi were obtained from soil closely adhered to root surfaces (rhizoplane, 0 distance) of lupine seedlings and the lowest from soil most distant from the roots (80 mm). From the surface of roots outward the rhizosphere effect was very pronounced to a distance of 3 mm, but was still evident at a distance of 18 mm. No counts were made between 18 and 80 mm.

The latter distance was considered as the non-rhizosphere soil (control), since in preliminary studies no differences were found between samples taken at this distance and others taken from unplanted soil.

Quantitative and qualitative determinations of fungi at various distances from roots of healthy seedlings indicated that certain species were preferentially encouraged by the roots, whereas others

TABLE 2

Five representative species of fungi (numbers per g of oven-dry soil) at various distances from the root surface of lupine seedlings grown in unamended Elsinboro sandy loam

Distance from root surface (mm)	*Aspergillus ustus*	*Cylindrocarpon radicicola*	*Fusarium oxysporum*	*Paecilomyces marquandii*	*Trichoderma viride*
0	5,650	4,940	5,330	9,000	5,900
0–3	3,360	0	2,500	2,800	22,100
3–6	2,920	0	2,800	1,600	6,900
9–12	2,880	0	4,480	1,500	14,600
15–18	2,270	0	4,100	0	7,300
80	1,000	0	5,070	0	11,100
R/S ratio	5.7		1.1		0.5

were apparently unaffected (Table 2). The rhizosphere effect depended on both the distance from the root surface and the individual fungus involved. For instance, *Cylindrocarpon radicicola* Wr. and *Paecilomyces marquandii* (Mass.) Hughes were very abundant in the rhizoplane ,but their numbers fell off sharply with distance from the root surface. Peterson [15] showed that Cylindrocarpon often predominated on the root surfaces of healthy red clover and wheat, but this predominance depended upon soil type. Evidently, whether Cylindrocarpon is a rhizophilic fungus depends somewhat upon soil conditions. *Aspergillus ustus* (Bain.) Thom and Church was more predominant at the root surface than in the soil distant from roots, but a rhizosphere effect was still evident 18 mm from the root surface. *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen and *Trichoderma viride* Pers. ex Fries, on the other hand, were not affected by the plant roots under the conditions of these experiments. During all repetitions in both soils *T. viride* occurred in higher numbers away from the root surface than in the rhizoplane.

The genus Penicillium comprised more than 50 per cent of the

total fungal counts. Peterson [15] found that the activity of Penicillium on wheat roots was confined mainly to the seedling stage. The plants in the experiments described herein were sampled at the seedling stage. The effect of lupine roots on Penicillium varied with the species. For instance, *Penicillium piscarium* Westl. was 46.7 times as abundant in the rhizoplane as 80 mm from the root surface (Table 3). On the other hand, *Penicillium lilacinum* Thom had a rhizosphere/soil (R/S) ratio of only 3.3. Genera like *Alternaria, Cephalosporium, Chaetomium, Cladosporium, Gliocladium, Humicola, Mucor, Myrothecium, Phoma, Rhizopus, Scopulariopsis,* and *Stysanus* were less abundant and were not included in Tables 2 and 3.

TABLE 3

Penicillium spp. (numbers per g of oven-dry soil) at various distances from the root surface of lupine seedlings grown in unamended Elsinboro sandy loam

Distance from root surface (mm)	Total Penicillium	*Penicillium piscarium*	*Penicillium lilacinum*
0	253,000	238,000	2,900
0–3	52,200	16,800	850
3–6	47,200	11,100	3,000
9–12	44,500	6,300	1,500
15–18	40,100	5,900	5,600
80	28,300	5,100	700
R/S ratio	9.0	46.7	3.3

Extent of rhizosphere of blue lupine in amended soil

Decomposable plant materials added to soil usually suppress the rhizosphere effect (Lochhead [13]). Work done on this subject, however, dealt mostly with bacteria in the rhizosphere *vs.* soil distant from roots. In the experiment reported herein (carried out twice) an attempt was made to enumerate fungi in the rhizoplane and at various distances from the root surface of lupine seedlings grown in Elsinboro sandy loam that had been amended with ground corn stover either non-enriched or enriched with NH_4NO_3. The amendments were added to the soil 28 days before planting. Samples from various distances from the root surface were taken with the microsampler 33 days after planting of the lupines.

No significant differences were found between fungal populations in the rhizoplane (0 distance) of lupine seedlings and those in soil taken up to 18 mm from the root surface (Table 4). Corn stover

TABLE 4

Fungi (thousands per g of oven-dry soil) at various distances from root surface of lupine seedlings grown in unamended and amended Elsinboro sandy loam

Distance from root surface (mm)	Unamended soil		Soil amended with corn stover	
	No NH_4NO_3	NH_4NO_3 added	No NH_4NO_3	NH_4NO_3 added
0	176	147	2480	3110
0–3	176	128	2140	3240
3–6	167	181	2310	3030
9–12	139	174	2410	2950
15–18	138	120	2290	2900
80	123	142	2680	2970
R/S ratio	1.4	1.0	0.9	1.0

without NH_4NO_3 reduced, to less than unity, the overall R/S ratios of fungi.

Detailed studies of individual rhizoplane and soil fungi (Table 5) indicated that in most instances in both the amended and the unamended soils there was an effect manifested by greater fungal counts in rhizoplane samples than in corresponding control soil samples. Species of *Penicillium* in general (*P. piscarium* in particular) and *Mortierella isabellina* Oud. were preferentially encouraged in the rhizoplane. A stimulation of *T. viride* was apparent only when NH_4NO_3 had been added to soil in the presence or absence of corn stover. Although the overall R S ratios (Table 4) did not indicate a

TABLE 5

The effect of addition of nitrogen and corn stover to Elsinboro sandy loam on the numbers of some fungi (thousands per g of oven-dry soil) in the rhizoplane of lupine seedlings and in soil distant from roots

Fungus	Unamended soil				Soil amended with corn stover			
	No NH_4NO_3		NH_4NO_3 added		No NH_4NO_3		NH_4NO_3 added	
	R *	S *	R	S	R	S	R	S
Aspergillus spp.	8	5	7	6	77	102	124	76
Fusarium spp.	5	5	2	4	40	49	25	24
Humicola brevis	6	2	5	4	1370	1210	975	1220
Penicillium spp.	88	53	82	74	584	334	823	584
Penicillium piscarium	14	5	13	9	122	47	339	96
Trichoderma viride	11	19	6	5	41	65	97	74
Mortierella isabellina	0	0	0	0	67	27	295	143

* Samples: R, rhizoplane; S, non-rhizosphere soil (control).

fungal stimulation in the rhizoplane, the numbers of some individual fungi were increased in the rhizoplane by some treatments and decreased by others (Table 5).

The isolation of *M. isabellina* from soil and rhizoplane of lupines grown in soil amended with corn stover only indicated that it is possible to bring about changes in the fungal flora by selective soil treatments. Katznelson [10] and Katznelson and Richardson [12], working with other crops, pointed out a definite shift in the rhizosphere micro-flora by selective soil treatments.

From Table 5 it appears that *Humicola brevis* (Gilman and Abbott) Gilman was markedly stimulated in both the soil and the rhizoplane of lupines grown in Elsinboro sandy loam amended with ground corn stover. *H. brevis* was identified easily on the original dilution plates by its elevated grey colony center, a black cir-

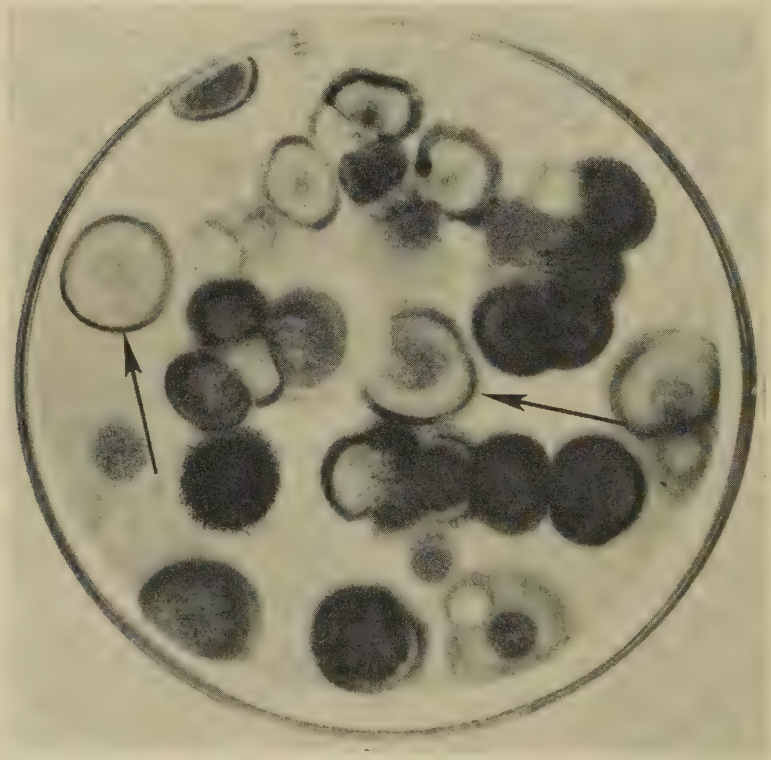

Plate 2. Colonies of *Humicola brevis* (indicated by arrows) on a dilution plate of V-8 juice-dextrose-yeast extract agar.

cumferential zone of intensive sporulation (Plate 2), and characteristic aleurospores which do not exceed 10μ in diameter.

Effect of nitrogen and particle size of corn stover on soil and rhizoplane fungi

Nitrogen-enriched and non-enriched corn stover of the following 3 particle sizes was added to Elsinboro sandy loam: small, ground in a Wiley mill to pass through a 1-mm screen, medium, cut by hand, 0.3 to 0.5 cm long; large, cut by hand, 1.0 to 1.3 cm long. Because of the large size of corn stover particles in the "medium" and "large" classes, the microsampler was not used in this experiment. Soil and rhizoplane samples were taken by the conventional method of sampling described in Materials and Methods.

The overall soil and rhizoplane fungal counts (Table 6) suggested that amendment particle size appreciably affects the numbers of fungi in soil and in the rhizoplane of lupine seedlings. Regardless of nitrogen the small particle size was associated with the highest fungal counts. The amended soil had 5 to 6 times as many fungi as the unamended soil. Again the dry plant material added to soil with or without nitrogen increased the numbers of fungi in the non-

TABLE 6

The effect of nitrogen and size of particles of corn stover added to Elsinboro sandy loam on the numbers of fungi (thousands per g of oven-dry soil) in the rhizoplane of lupine seedlings and in soil distant from roots

Particle size and nitrogen	Rhizoplane	Non-rhizosphere soil	R/S ratio
Small			
No NH_4NO_3	1810	1190	1.5
NH_4NO_3 added	1680	1060	1.6
Medium			
No NH_4NO_3	1640	612	2.7
NH_4NO_3 added	688	323	2.1
Large			
No NH_4NO_3	1390	536	2.6
NH_4NO_3 added	1360	392	3.5
Unamended soil			
No NH_4NO_3	877	216	4.1
NH_4NO_3 added	801	258	3.1

TABLE 7

Abundance of some fungi (thousands per g of oven-dry soil) in lupine rhizoplane as affected by size of particles of N-enriched corn stover added to Elsinboro sandy loam 25 days before planting

Fungal genus	Particle size of N-enriched corn stover			N-enriched control soil
	Small	Medium	Large	
Aspergillus	11	5	170	9
Fusarium	56	38	162	5
Gliocladium	1	1	1	4
Humicola	28	94	253	2
Myrothecium . . .	5	17	0	0
Penicillium	909	266	524	589
Trichoderma . . .	14	34	13	34

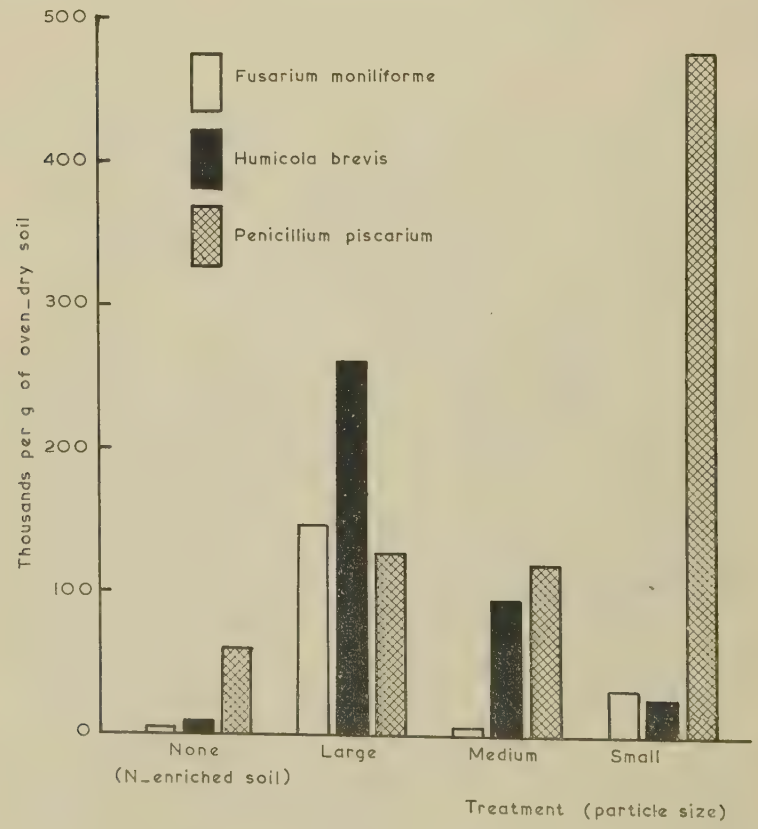

Fig. 2. Numbers of *Fusarium moniliforme*, *Humicola brevis*, and *Penicillium piscarium* in lupine rhizoplane as affected by particle size of N-enriched corn stover added to Elsinboro sandy loam 25 days before planting.

rhizosphere soil more than in the rhizoplane thus narrowing the R/S ratios.

The effect of particle size on the distribution of the most abundant genera in the rhizoplane of lupines was striking (Table 7). Aspergillus, Fusarium, and Humicola were stimulated more in the rhizoplane of lupine seedlings grown in soil amended with large particles than in the rhizoplane of seedlings grown in soil amended with medium or small particles with or without nitrogen. On the other hand Myrothecium, Penicillium, and Trichoderma were stimulated more by small particles than by large ones.

Counts of the 3 most prevalent species indicated the importance of particle size in affecting abundance of fungi in soil and in the rhizoplane of lupines. For instance, *Fusarium moniliforme* Scheld. emend. Snyder and Hansen and *H. brevis* were abundant in the rhizoplane of seedlings grown in soil amended with large particles of corn stover, whereas *P. piscarium* was prevalent in the rhizoplane of plants in soil amended with small particles (Fig. 2). With respect to *H. brevis* a 3-fold increase in counts was observed in the non-rhizosphere soil amended with small particles (nitrogen-enriched or non-enriched) over those with medium or large particles. Additional stimulation of *H. brevis* was brought about by nitrogen in the amended but not in the unamended soil (Fig. 3).

Effect of various plant materials and nitrogen on soil and rhizoplane fungi

Fungal counts from soil and rhizoplane of healthy blue lupines grown in soil amended with nitrogen-enriched and non-enriched finely ground dry mature plant materials are shown in Table 8. All plant materials (with or without supplemental nitrogen) stimulated fungi more than soil that had received nitrogen only. The increase attributed to the plant materials added to soil was more pronounced in the non-rhizosphere soil than in the rhizoplane. This disproportional increase of fungi in soil than in the rhizoplane narrowed the R/S ratios. The greatest increase in fungal counts in both the rhizoplane and the non-rhizosphere soil was caused by nitrogen-enriched corn stover and the smallest by soybean meal.

Large increases were observed in counts of Humicola and Penicillium in soil and in the rhizoplane of lupines grown in Elsinboro sandy loam amended with nitrogen-enriched barley

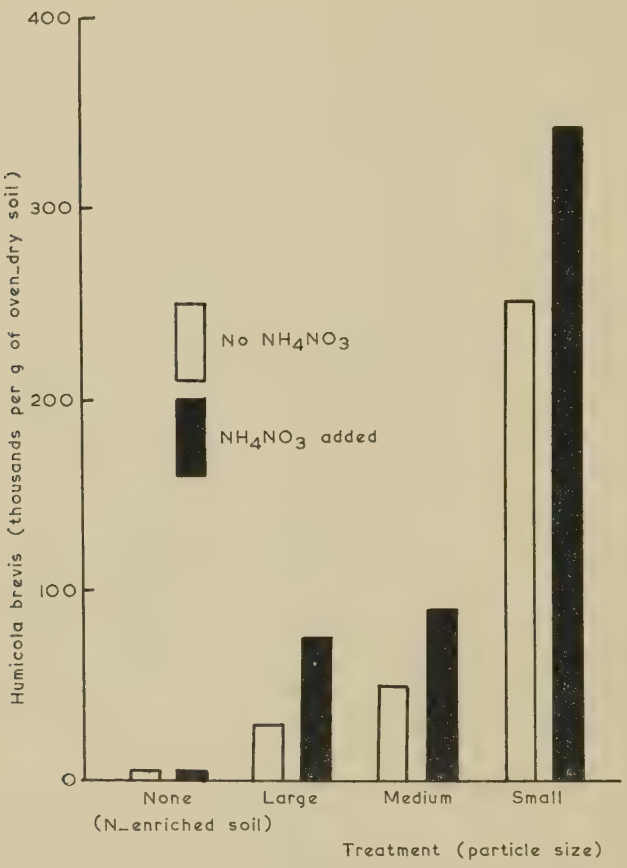

Fig. 3. Numbers of *Humicola brevis* in non-rhizosphere soil as affected by NH_4NO_3 and particle size of corn stover added to Elsinboro sandy loam 45 days before sampling.

straw, corn stover, or soybean meal (Table 9). Humicola was stimulated more by barley straw than by the other 2 plant materials, whereas Penicillium was stimulated more by soybean meal than by barley straw or corn stover. In the soybean meal-treated soil, *P. piscarium* comprised approximately 70 per cent of the total *Penicillium* spp. *P. lilacinum* comprised about 20 per cent of the total *Penicillium* spp., but the total counts of *P. lilacinum* (396,000 per g oven-dry soil) represented a marked increase over the unamended controls as well as over barley straw and corn stover. *M.*

TABLE 8

The effect of nitrogen and dry plant materials added to Elsinboro sandy loam on the numbers of fungi (thousands per g of oven-dry soil) in the rhizoplane of lupine seedlings and in soil distant from roots

Plant material and nitrogen *	Rhizoplane	Non-rhizosphere soil	R/S ratio
Barley straw			
No NH_4NO_3	899	2,210	0.4
NH_4NO_3 added	1,360	2,730	0.5
Corn stover			
No NH_4NO_3	1,270	1,920	0.7
NH_4NO_3 added	1,480	3,790	0.4
Soybean meal			
No NH_4NO_3	704	1,480	0.5
NH_4NO_3 added	494	1,380	0.4
Unamended soil			
No NH_4NO_3	154	145	1.1
NH_4NO_3 added	162	153	1.1

* Added to soil 28 days before planting.

isabellina was stimulated in the rhizoplane of lupines grown in soil amended with barley straw or corn stover, but it was absent in the rhizoplane of lupines grown in unamended soil or in soil amended with soybean meal. An appreciable reduction in the total population of Fusarium was also evident in both the non-rhizosphere soil and

TABLE 9

The effect of dry plant materials added to Elsinboro sandy loam on the numbers of some fungi (thousands per g of oven-dry soil) in the rhizoplane of lupine seedlings

Fungus	Plant material added to soil			
	Barley straw	Corn stover	Soybean meal	None (control soil)
Aspergillus spp.	12	54	0	10
Fusarium spp.	6	20	2	5
Gliocladium spp.	3	7	0	0
Humicola spp.	954	499	284	2
Penicillium spp.	508	653	1,970	170
Penicillium piscarium	260	258	1,390	90
Penicillium lilacinum	5	23	396	0
Trichoderma viride	5	4	2	7
Mortierella isabellina	409	138	0	0

in the rhizoplane of lupines grown in soil amended with soybean meal.

Effect of plant materials on root-surface fungi

The roots of lupines from which rhizoplane samples had been prepared in the previous experiments involving nitrogen-enriched and non-enriched barley straw, corn stover, and soybean meal were used to determine the frequency of occurrence of various fungi on the root surfaces. The results obtained by the use of procedures outlined in Materials and Methods are expressed as per cent frequency of occurrence obtained on the basis of 25 washed root pieces, replicated 4 times on 4 media (Table 10).

The most outstanding feature of this experiment was the presence on the root surface of apparently healthy lupine seedlings of such fungi as Cephalosporium, Pythium, Rhizoctonia, Sporotrichum, Thielavia, Verticillium, and 2 sterile forms. Most of these fungi are not normally isolated by the dilution-plate method. Cephalosporium, Sporotrichum, Verticillium, and 2 sterile forms were increased by the plant materials and especially by corn stover and soybean meal. Results with nitrogen-enriched plant materials were essentially similar to those without nitrogen and are not included

TABLE 10

The effect of dry plant materials added to Elsinboro sandy loam on the predominant root-surface fungi of lupine seedlings

Fungal genus	Frequency of occurrence (%) with specified plant material added to soil			
	Barley straw	Corn stover	Soybean meal	None (control soil)
Cephalosporium	2.8	16.0	13.6	0.0
Chaetomium	1.3	2.3	0.0	2.3
Cylindrocarpon	1.2	2.8	0.0	1.2
Fusarium	20.0	22.8	40.0	42.4
Gliocladium	14.9	20.0	36.6	20.0
Humicola	57.2	44.0	54.4	0.0
Paecilomyces	13.6	5.2	5.2	8.0
Pythium	13.3	12.0	25.2	20.0
Rhizoctonia	1.2	0.0	0.0	1.2
Sporotrichum	12.0	13.2	9.2	5.2
Thielavia	2.0	1.0	1.2	1.8
Verticillium	6.4	14.8	29.2	5.7
Sterile hyaline	9.2	14.9	18.4	6.8
Sterile brown	8.4	5.6	12.0	5.2

in Table 10. The genus Pythium was isolated from roots grown in both amended and unamended soil. Rhizoctonia was isolated from roots grown in the unamended soil as well as in soil amended with barley straw.

Of the genera normally isolated with the dilution-plate method, Humicola was the only one stimulated on the root surface by all plant materials. Gliocladium was stimulated by soybean meal, whereas Chaetomium and Cylindrocarpon were suppressed. Although soybean meal reduced Fusarium in the rhizoplane of lupines (Table 9), the same plant material did not reduce the population of Fusarium on the root surface of the seedlings. Barley straw and corn stover reduced Fusarium at the root surface by 50 per cent.

DISCUSSION

In the foregoing studies an attempt was made to determine the extent of the zone of lupine root effect on bacteria, streptomycetes, and fungi. The determination of these micro-organisms by the dilution-plate method has several inherent limitations. Some of the most outstanding ones are the lack of information on the precise origin of colonies (whether from spores or mycelial fragments) and the possibility that some important organisms may not be isolated from soil. Although methods of desiccation indicate that colonies developing from spores represent only small fractions of the total rhizosphere counts (Timonin [23], Agnihothrudu [3]), it is generally agreed that the dilution-plate method in all its variations gives essentially only a survey of actively sporing fungi. Despite these limitations, this method enables a more or less approximate determination of a portion of the soil fungus population. Burges [4] in his recent book criticized the dilution-plate method, but he concluded that "despite all the disadvantages of the dilution method, it has been of great value, and if due regard is paid to the known limitations it is a most useful and productive means of investigating certain aspects of soil microbiology".

For most of the studies reported herein, the method proposed by Harley and Waid [7] for determining actively growing fungi on plant roots was not useful since it reveals only the fungal population that remains attached to the root surface after a number of

washings. This method cannot be used as a means of determining the presence of fungal mycelia at various distances from the roots. Although the hyphal isolation method described by Warcup [26] is of great value in determining actively growing fungi in soil, it is too elaborate for use in extensive quantitative and qualitative investigations.

The study of the relative abundance of bacteria, streptomycetes, and fungi in the rhizoplane and at various distances from the roots of lupine seedlings indicated that the rhizosphere effect was not confined to the rhizoplane and to soil immediately adjacent to the roots, but it extended at least 18 mm from the root surface. The extent of the rhizosphere effect to a distance of 18 mm must be attributed to root exudates diffusing out from the root rather than to sloughed-off root cells.

With fungi, the root effect depended not only on the distance from the roots, but also on the fungal species involved and the soil treatment. For instance, *Penicillium* spp. were 9 times more prevalent in the rhizoplane than 80 mm away from it. Specifically, however, *P. piscarium* and *P. lilacinum* were 46.7 and 3.3 times, respectively, more prevalent in the rhizoplane than 80 mm from the roots. This and other examples reported in the foregoing experiments indicated that generalizations regarding the root effect on individual fungi may not be warranted from overall rhizosphere/soil ratios. Only a few studies on the effect of roots on individual fungi were previously reported (Agnihothrudu [2], Katznelson and Richardson [12], Peterson [15], Timonin [23]).

The rhizosphere effect exerted by roots of lupine seedlings grown in unamended soil was less pronounced when various plant materials and nitrogen were added to the same soil. The reduction was attributed to a stimulatory effect of the plant materials and nitrogen on soil fungi at all distances from the rhizoplane rather than to a suppressing effect on the rhizoplane fungi. Ground corn stover with or without nitrogen provided an enormous surface for microbial activity which overshadowed the rhizosphere effect attributed to root exudates or to sloughed-off cells. When individual fungal genera and species were determined in amended soil, however, there was a marked rhizosphere effect on some fungi but not on others, and this effect was modified by the presence or absence of nitrogen. A reduction of the rhizosphere effect was discussed by

others (Adati [1], Lochhead [13]), and attributed to the fact that root excretions were effective as stimulants of micro-organisms under conditions of limited nutrients (Peterson [15]).

The rhizosphere effect in unamended Elsinboro sandy loam without nitrogen was very low in 2 experiments (Table 4, 8) and high in 2 others (Table 1, 6). Since methodology, soil type and age of plants were the same in all 4 experiments, the observed differences may be attributed to the fact that the experiments in which low R/S ratios were observed were carried out during warm, long summer days, while the experiments in which high R/S ratios were observed were carried out during relatively cool and short days. Variations in day length and temperature may also explain differences observed in rhizosphere numbers of *P. piscarium* in unamended soil (Table 3, 5). Low rhizosphere numbers of this fungus were associated with long, warm days.

Soil and rhizoplane fungi were affected not only by various plant materials and nitrogen added to soil, but also by the particle size of the plant material used. Ground corn stover appreciably increased the overall counts of fungi above those obtained when medium or large particles were used. The small particle size, however, was not the most favorable for all fungi examined. Aspergillus, Fusarium, and Humicola were stimulated by the large particles, whereas Myrothecium, Penicillium, and Trichoderma were stimulated by the small ones. The reasons for this differential stimulation were not brought out by these studies. It is possible, however, that the 3 fungi stimulated by the large particles are able to penetrate these particles and use nutrients unaccessible to other organisms lacking this ability.

The effect of decomposing barley straw, corn stover, or soybean meal was also observed on some root-surface fungi determined by a method similar to that described by Harley and Waid [7]. Fungi not normally seen on dilution plates, (Pythium, Rhizoctonia, Thielavia, Verticillium, sterile mycelia) as well as the abundantly sporing fungi, were differentially affected on the root surface by the decomposing plant materials. Studies on the effect of soil treatments on the fungal flora of the root surface are limited. Since most of the intimate associations of plants and fungi occur at the root surface, this fungal microhabitat needs to be studied in greater detail.

SUMMARY

To determine the extent and nature of the rhizosphere of *Lupinus angustifolius* (blue lupine) a microsampler was built whereby serial soil microsamples 3 mm in diameter and 10 to 15 mm long could be obtained simultaneously from the surface of the roots outward to a distance of 21 mm.

In unamended soils the highest number of micro-organisms was obtained from the rhizoplane and the lowest from soil 80 mm from the roots. From the surface of the roots outward the rhizosphere effect was very pronounced to 3 mm, but was still evident at 18 mm. Determinations of fungi at various distances from roots of healthy lupine seedlings indicated that certain fungal species and genera were preferentially encouraged by the roots, whereas others were apparently unaffected. The degree of the rhizosphere effect depended on the distance from the roots and on the individual fungi.

In Elsinboro sandy loam amended with various plant materials, unsupplemented or supplemented with NH_4NO_3, no significant differences were found in numbers of fungi between the rhizoplane and soil samples at various distances from the roots. Soils that received plant materials stimulated fungi more than soils that received nitrogen only. The plant materials reduced the overall rhizosphere/soil ratios of fungi. When individual fungal genera and species were considered, a definite rhizosphere effect was still observed, but this depended on the kind of plant material added to soil and on the fungus and its habitat.

The particle size of corn stover added to Elsinboro sandy loam appreciably affected numbers of fungi in the soil and in the rhizoplane. The small particle size was associated with the highest total fungal counts. The effect of particle size of corn stover was altered by the presence or absence of supplemental nitrogen and varied with the fungal species.

Quantitative and qualitative changes in fungi on the root surfaces of lupine seedlings were brought about by barley straw, corn stover, soybean meal, and nitrogen added to soil 28 days before planting. Cephalosporium, Sporotrichum, Verticillium, and 2 sterile fungi were stimulated by all the plant materials, expecially by corn stover and soybean meal. Fusarium was reduced by soybean meal in the soil and in the rhizosphere of lupines but not on the root surface. Changes in root-surface fungi depended on the plant material added.

ACKNOWLEDGEMENTS

The authors are indebted to Drs. C. W. Hesseltine and C. R. Benjamin, Fermentation Laboratory, Northern Utilization Research and Development Division, Peoria, Illinois, for assistance in identifying some fungi.

Received April 11, 1960

REFERENCES

1 Adati, M., Untersuchungen über die Rhizosphäre der Pflanzen. J. Soc. Trop. Agr. (Taiwan) **11**, 57–65 (1939).

2 Agnihothrudu, V., Soil conditions and root diseases. VIII. Rhizosphere microflora of some of the important crop plants of South India. Proc. Indian Acad. Sci. B **37**, 1–13 (1953).

3 Agnihothrudu, V., State in which fungi occur in the rhizosphere. Naturwissenschaften **42**, 515–516 (1955).

4 Burges, A., Micro-organisms in the Soil. Hutchinson and Co. Ltd. (Publishers), London (1958).

5 Clark, F. E., Experiments toward the control of the take-all disease of wheat and the Phymatotrichum root rot of cotton. U.S. Dept. Agr. Techn. Bull. No. **835** (1942).

6 Clark, F. E., Soil microorganisms and plant roots. Advances in Agron. **1**, 241–288 (1949).

7 Harley, J. L. and Waid, J. S., A method of studying active mycelia on living roots and other surfaces in the soil. Brit. Mycol. Soc. Trans. **38**, 104–118 (1955).

8 Hiltner, L., Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngen und Brache. Arb. deut. landw. Ges. **98**, 59–78 (1904).

9 Ishizawa, S., Suzuki, T., Sato, O., and Toyoda, H., Studies on microbial population in the rhizosphere of higher plants with special reference to the method of study. Soil and Plant Food (Tokyo) **3**, 85–94 (1957).

10 Katznelson, H., The "rhizosphere effect" of mangels on certain groups of soil microorganisms. Soil Sci. **62**, 343–354 (1946).

11 Katznelson, H., Lochhead, A. G., and Timonin, M. I., Soil microorganisms and the rhizosphere. Botan. Rev. **14**, 543–587 (1948).

12 Katznelson, H. and Richardson, L. T., Rhizosphere studies and associated microbiological phenomena in relation to strawberry root rot. Sci. Agr. **28**, 293–308 (1948).

13 Lochhead, A. G., Rhizosphere microorganisms in relation to root-disease fungi. *In:* Holton, C. S., *et al.* Eds. Plant Pathology: Problems and Progress 1908–1958 p. 327–338. University of Wisconsin Press, Madison (1959).

14 Papavizas, G. C. and Davey, C. B., Evaluation of various media and antimicrobial agents for isolation of soil fungi. Soil Sci. **88**, 112–117 (1959).

15 Peterson, E. A., Observations on fungi associated with plant roots. Can. J. Microbiol. **4**, 257–265 (1958).

16 Peterson, E. A., Seed-borne fungi in relation to colonization of roots. Proc. Intern. Botan. Congr. 9th Congr. Montreal **2**, 298 (1959).

17 Ramachandra-Reddy, T. K., Foliar spray of urea and rhizosphere microflora of rice (*Oryza sativa* L.). Phytopathol. Z. **36**, 286–289 (1959).

18 Rovira, A. D., Some quantitative and qualitative aspects of the rhizosphere. Australian Conf. Soil Sci. (Adelaide) **1**, 1–7 (1953).

19 Starkey, R. L., Some influences of the development of higher plants upon the microörganisms in the soil: IV Influence of proximity to roots on abundance and activity of microörganisms. Soil Sci. **32**, 367–393 (1931).

20 Starkey, R. L., Interrelationships between microörganisms and plant roots in the rhizosphere. Bacteriol. Rev. **22**, 154–172 (1958).

21 Thom, C. and Humfeld, H., Notes on the association of microörganisms and roots. Soil Sci. **34**, 29–36 (1932).

22 Timonin, M. I., The interaction of higher plants and soil micro-organisms, I Mi-

crobial population of rhizosphere of seedlings of certain cultivated plants. Can. J. Reserach C **18**, 307–317 (1940).

23 Timonin, M. I., The interaction of higher plants and soil micro-organisms: II Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne diseases. Can. J. Research C **18**, 444–456 (1940).

24 Timonin, M. I., The interaction of higher plants and soil micro-organisms: III Effect of by-products of plant growth on activity of fungi and actinomycetes. Soil Sci. **52**, 395–413 (1941).

25 Waksman, S. A., Microbiological analysis of soil as an index of soil fertility, II. Methods of the study of numbers of microörganisms in the soil. Soil Sci. **14**, 283–298 (1922).

26 Warcup, J. H., Isolation of fungi from hyphae present in soil. Nature **175**, 953–954 (1955).

27 West, P. M. and Hildebrand, A. A., The microbiological balance of strawberry root rot soil as related to the rhizosphere and decomposition effects of certain cover crops. Can. J. Research C **19**, 199–210 (1941).

Plant and Soil XIV, no 3 May 1961

EFFECT OF NUTRITIONAL STRESS ON PLANT COMPOSITION

III. CHARACTERIZATION OF THE PECTIC SUBSTANCES IN NORMAL AND SULFUR-DEFICIENT ALFALFA

by E. A. McCOMB and V. V. RENDIG

Department of Soils and Plant Nutrition
University of California, Davis, California

INTRODUCTION

Some effects of sulfur nutrition on the quantity and distribution of reducing and non-reducing sugars and amide-N fraction and on the methionine content of alfalfa plants have been the subject of previous reports [8 9 10]. Since a considerable portion of the sulfur required by plants is needed to form methionine [13], and since methionine has been assigned a role in methylation in biological systems, the possibility of an indirect effect of sulfur nutrition on the characteristics of the pectic substances of alfalfa was explored.

Summarizing the work of others, Jansen *et al.*[2] have reported that the middle lamella of plant cells is characterized by a proportionately large content of pectic substances, and the primary cell wall of coleoptile cell is composed of 25 to 40 per cent α-cellulose, 8 to 12 per cent pectic substances, and 10 to 20 per cent waxes. Lagowski *et al.*[3] have investigated the pectic substances in alfalfa and determined the methoxyl value of an isolated fraction.

The interchange between the methyl group of methionine and the pectic substances has been shown by several investigators. The methyl ester group of methionine has been shown to contribute at least in part to the methyl ester group of pectin [6 11]. The biosynthesis of pectinic acid methyl esters through transmethylation from methionine has been reported in radish plants [12]. In the pectin metabolism of growing cell walls a relationship has been demon-

strated between methionine and the methyl esterification of pectic substances [2].

The possibility that the nutritional stress caused by a deficiency of sulfur would directly affect the pectin metabolism and more particularly the type of pectic substances elaborated by the tissue was investigated by the characterization of the pectic substances in a marc prepared from alfalfa plants grown with an adequate and a deficient sulfur supply as the only variable. Particular interest was taken in the degree of methyl esterification, however, acetyl determinations were also made. The presence of acetyl esters of pectic substances has not been recognized to any appreciable extent owing to the relative insolubility of acetylated polymers *in situ*, and because methods of extraction and treatment of the pectic substances prior to analysis have probably contributed to the loss of this ester.

From the data some conclusions are drawn regarding the effect of nutritional stress on the pectic substances formed in plant tissue and on the characteristics of these substances.

PROCEDURE AND METHODS

Plants from cuttings from a single clone of Caliverde variety alfalfa were grown at two levels of sulfur nutrition (8.0 and 0.02 ppm S) using a modified nutrient-culture technique previously described [9]. Three replicates containing 4 to 5 cuttings each were grown at each level of sulfur. Plants grown at the 8-ppm sulfur level yielded normal healthy shoots in contrast to stunted, chlorotic plants at the 0.02-ppm level. Growth at the 0.02-ppm sulfur level was sufficient, however, to yield enough plant material for analysis.

Shoots of approximately the same age (about 1 month) were harvested, the leaf-petiole parts immediately separated from the stems and both fractions transferred directly into boiling 95% ethyl alcohol. The roots were also separated, washed and placed directly into boiling 95% alcohol. By taking into account the water content of the plant material, the volume of 95% alcohol used was adjusted to obtain a final concentration of about 80% alcohol. A marc was prepared from the residue from the alcohol extract by the procedure described by Gee *et al.*[1]. The dry marc sample was ground to pass a 40-mesh screen and the total anhydrouronic acid (AUA) determined by the pectase–carbazole method [5], the methoxyl content determined by titration [7], and calculated by correcting for free carboxyl and acetyl, and the acetyl determined by the colorimetric hydroxamic acid method [4]. The reliability of the procedures used was checked by comparing the AUA determined colorimetrically with that found by titration (free COOH plus COOH esterified with OCH_3). Reasonable agreement was found and it was

assumed that there was negligible interference in the titration procedure owing to alkalinity of the ash or other titratable non-pectic substances remaining in the marc. The degree of methyl esterification was calculated as follows:

$$\text{Percentage esterification} = \frac{\text{esterified uronic acid carboxyls} \times 100}{\text{total uronic acid carboxyls}}$$

RESULTS AND DISCUSSION

The data in Table 1 show that the percent total pectin (as AUA) found in the marc prepared from the sulfur-deficient plant tissues was consistently greater than that in the marc from normal plants. The stem fractions show the largest difference and the variations in percent pectin in the fractions were more pronounced in the normal plants. It is interesting to note also that the greater percent AUA in

TABLE 1

Characterization of the pectic substances in normal (8 ppm S) and sulfur-deficient (0.02 ppm S) alfalfa.
Percentages expressed on basis of dry weight of the marc

Plant part	AUA %	Acetyl %	Methoxyl %	Degree of esterification %
0.02 ppm sulfur				
Leaf-petiole	16.8	2.0	1.6	57
Stem	15.7	3.4	0.9	39
Root	12.4	2.4	0.9	48
8.0 ppm sulfur				
Leaf-petiole	14.5	1.5	1.3	40
Stem	8.7	3.3	0.6	33
Root	10.8	2.3	0.8	44

the pectic substances of sulfur-deficient plants than of normal plants is in contrast to a decrease in reducing and non-reducing sugars in these tissues [10]. Of the three plant parts, the effect of sulfur nutrition on the degree of methyl esterification of the AUA in the marc was most evident in the leaf-petiole fractions where 57 per cent esterification was found for sulfur-deficient plants as compared with 40 per cent for normal plants. The somewhat greater degree of methyl esterification of the pectic substances in sulfur-deficient plants is consistent with previous findings [8] that the percentage of methionine in the organic sulfur fraction was slightly greater in the

sulfur-deficient plants than in those grown in the same soil after large additions of gypsum. The acetyl content of the pectic substances was not changed by sulfur nutrition, but considerable variation was found in the analysis of the different plant parts. In normal tissue the pectic substances in the stems contained 3.3 per cent acetyl in contrast to 1.5 per cent in leaf-petiole and 2.3 per cent in the root tissue and these values are similar to those found in the corresponding parts of the sulfur-deficient plants.

The data concerning the effect of sulfur nutrition on the metabolism of pectic substances and their characteristics may indicate that the pectic substances in sulfur-deficient plants are formed normally, but that their distribution and catabolism are abnormal.

A preliminary investigation of the total pectic substances in phosphorus-deficient tissue as compared with normal alfalfa showed substantially the same trend as that found for sulfur. The leaf-petiole and root fractions contained nearly the same percent AUA, whereas the deficient stems contained 1.5 times the AUA of the normal stems. Although the pectic substances in the phosphorus-deficient tissues were not characterized, the gross picture would seem to indicate that the pattern of total AUA is the same when either sulfur or phosphorus was deficient.

These data point out the necessity of studying plant parts rather than whole plants. This would seem particularly necessary when pectic substances are to be characterized and their characterization related to structural or physiological processes.

A study of pectic enzyme activities in the nutritionally deficient and normal plants was not undertaken, but such an investigation is planned and this may help to clarify somewhat the data found in this paper.

SUMMARY

Pectic substances were determined and characterized in marc prepared from leaf-petiole, stem, and root fractions of alfalfa grown in nutrient cultures containing adequate and inadequate sulfur. The percent pectin (as AUA) was somewhat greater in the sulfur-deficient tissues, the stem fractions showing the widest difference. The degree of methyl esterification of the pectic substances was not too dissimilar, however, the sulfur-deficient tissues appeared to be slightly more esterified, especially the leaf-petiole fraction. Acetyl values were not affected by the sulfur nutrition but consider-

able variance in percent acetyl was found in the different plant parts. The data is interpreted with respect to nutritional effects on the metabolism of pectic substances in plant tissue, and on the degree of methyl esterification of pectin in relation to methionine and sulphur nutrition.

Received July 23, 1960

REFERENCES

1 Gee, Mildred, McComb, Elizabeth A., and McCready, Rolland M., A method for the characterization of pectic substances in some fruit and sugar-beet marcs. Food Research **23**, 72–75 (1958).

2 Jansen, Eugene F., Jang, Rosie, Albersheim, Peter, and Bonner, James, Pectic metabolism of growing cell walls. Plant Physiol. **35**, 87–97 (1960).

3 Lagowski, Jeanne M., Sell, Harold M., Huffman, C. F., and Duncan, C. W., The carbohydrates in alfalfa *Medicago sativa*. I. General composition, identification of nonreducing sugar and investigation of the pectic substances. Arch. Biochem. Biophys. **76**, 306–316 (1958).

4 McComb, E. A. and McCready, R. M., Determination of acetyl in pectin and in acetylated carbohydrate polymers. Hydroxamic acid reaction. Anal. Chem. **29**, 819–821 (1957).

5 McCready, R. M. and McComb, E. A., Extraction and determination of total pectic materials in fruits. Anal. Chem. **24**, 1986–1988 (1952).

6 Ordin, L., Cleland, R., and Bonner, J., Influence of auxin on cell wall metabolism. Proc. Natl. Acad. Sci. U.S. **41**, 1023–1029 (1955).

7 Owens, H. S., McCready, R. M., Shepherd, A. D., Shultz, T. H., Pippen, E. L., Swenson, H. A., Miers, J. C., Erlandsen, R. F., and Maclay, W. D., Methods used at Western Regional Research Laboratory for extraction and analysis of pectic materials. U.S. Dept. Agriculture, Bureau Agr. Ind. Chem., AIC – **340** (1952).

8 Rendig, V. V., Sulfur and nitrogen composition of fertilized and unfertilized alfalfa grown on sulfur-deficient soil. Soil Sci. Soc. Am. Proc. **20**, 237–240 (1956).

9 Rendig, V. V. and McComb, E. A., Effect of nutritional stress on plant composition: I. The interaction of added nitrogen with varying sulfur supply. Soil Sci. Soc. Am. Proc. **23**, 377–380 (1959).

10 Rendig, V. V. and McComb, E. A., Effect of nutritional stress on plant composition. II. Changes in sugar and amide nitrogen content of normal and sulfur-deficient alfalfa during growth. Plant and Soil **14**, 176–186 (1961).

11 Sato, C., Methyl group synthesis in plant metabolism. Thesis, Mich. State Univ. (1955).

12 Sato, Clifford S., Byerrum, Richard U., and Ball, Charles D., The biosynthesis of pectinic acid methyl esters through transmethylation from methionine. J. Biol. Chem. **224**, 717–723 (1957).

13 Steward, F. C., Thompson, J. F., Miller, F. K., Thomas, M. D., and Hendricks, R. H., The amino acids of alfalfa as revealed by paper chromatography with special reference to compounds labeled with S^{35}. Plant Physiol. **26**, 123–125 (1951).

Plant and Soil XIV, no 3 May 1961

UNCOUPLING OF PHOSPHORYLATION IN CHLORELLA BY 2,4-DICHLOROPHENOXYACETIC ACID

by RANDOLPH T. WEDDING and M. KAY BLACK

Department of Plant Biochemistry, University of California

INTRODUCTION *

The plant growth regulator 2,4-D has been reported to cause marked changes in the phosphorus metabolism of a number of species. It has also been observed to decrease P/O ratios in preparations of rat-liver [1] and soybean [4] mitochondria. However, the degree to which 2,4-D actually inhibits the formation of ATP and its effectiveness relative to such uncouplers as DNP have not been evaluated. The present report is of an attempt at such an evaluation.

EXPERIMENTS

Effects of 2,4-D on phosphate incorporation into nucleotides

The experimental material was *Chlorella pyrenoidosa*, Chick. (Emerson's strain) grown in nutrient solution under fluorescent lamps and concentrated as described previously [5]. The 2,4-D and DNP were from commercial sources, purified by two recrystallizations from hot water.

A suspension of Chlorella containing 0.2 ml of cells in 0.01 *M* K-phosphate buffer, pH 5.5, labelled with P^{32}, was incubated under lights in a shaking waterbath at 25° C for 20 hours. The cells were sedimented by centrifugation, and resuspended in 1 ml of boiling 80 per cent ethanol. The residue was separated centrifugally and extracted with 2 ml of hot 20 per cent ethanol. The two ethanol extracts were combined and the solvent removed at low temperature. Following concentration, the extract was streaked on a 2-inch

* Abbreviations: 2,4-D = 2,4-dichlorophenoxyacetic acid, DNP = 2,4-dinitrophenol, ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate.

strip of Schleicher and Schuell No. 589 (green label) filter paper and the chromatogram developed with a solvent consisting of ethanol/ammonia/water (80 5 15) for 30 hours. The portion of this strip containing radioactivity was cut into 7 one-inch strips, eluted with water, and the eluates rechromatographed separately in the same solvent. The strips were scanned for radioactivity, and the quantity of P^{32} present obtained by integrating the area under the recorded peaks of activity corresponding to ATP, ADP, and AMP plus inorganic orthophosphate. The AMP and inorganic phosphate are reported together because their separation was not adequate for satisfactory quantitative determination. Comparisons using charcoal adsorption methods indicate that most of the activity in this fraction is inorganic phosphate.

The effect of 2,4-D on P^{32}-incorporation into these compounds in Chlorella is shown in Table I, which summarizes an experiment of this type. It is seen that 10^{-4} *M* 2,4-D reduces the P^{32}-incorporation into ATP and ADP to about 30 per cent of that in untreated cells. AMP plus inorganic phosphate was much less affected, the amount in the cells being about 85 per cent of that in the controls. The total

TABLE 1

Effect of 2,4-D on the incorporation of $P^{32}O_4$ into nucleotides of Chlorella from 10^{-2} *M* PO_4-solution. Extraction by boiling ethanol and separation by chromatography in ethanol/ammonia/water. First chromatogram divided into 7 sections, eluted and the eluates rechromatographed separately. Activity determined by radioactive scan of the second strips. All figures in mμ atoms of phosphorus per sample of 0.2 ml of packed Chlorella cells

2,4-D concentration *M* (total acid)	ATP	ADP	AMP + Pi	Total
0	121	67	441	751
10^{-4}	37	23	375	530
10^{-2}	3	3	171	185

phosphorus, which includes several other fractions separated but not identified, was reduced by the highest concentration of 2,4-D to about 25 per cent of the untreated level. Similar results with respect to the effects of 2,4-D on the labelling of ATP and ADP have been obtained using cold $HClO_4$ extraction followed by adsorption of the nucleotides on charcoal and elution with 3 per cent ammonia in 50 per cent ethanol and separation of the eluted nucleotides by ionophoresis in 0.03 *M* K-citrate buffer at pH 6.0.

Comparison of 2,4-D and DNP effects on phosphate incorporation

Cell suspensions in K-citrate buffer with added $P^{32}O_4$ were incubated for 4

hours at 25° C with various concentrations of 2,4-D and DNP. A modification of Laties' method[2] was used to extract the nucleotides and separate nucleotide phosphate and inorganic phosphate. The cells were sedimented by centrifugation at 100 × g for 2 minutes and extracted for 5 minutes in 1 ml of 1 *N* $HClO_4$ with ultrasonic oscillation in an ice bath. The residue was sedimented centrifugally and the extract poured into a tube containing 25 mg of acid-washed Norite-A charcoal. The charcoal was suspended in the extract, collected by centrifugation, and the extract poured off. The cells were extracted three times, with each subsequent extract adsorbed separately on the same charcoal. The charcoal was rinsed with 1 ml 0.05 *M* Na-acetate buffer to remove inorganic phosphate, transferred to planchets and the P^{32}-radioactivity determined. Aliquots of the non-adsorbed fraction were also counted.

Preliminary studies using this technique showed that DNP at the same concentration as 2,4-D was a more effective inhibitor of the incorporation of P^{32} into nucleotides by Chlorella. Since it is known that the effectiveness of weak acid toxicants in solution is largely,

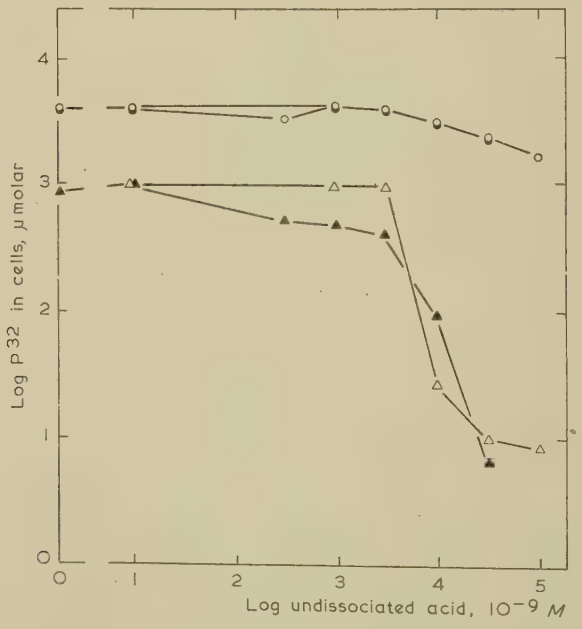

Fig. 1. Effects of 2,4-D and DNP in equal concentrations of undissociated acid on the incorporation of P^{32} into nucleotides of Chlorella. Circles = Pi ($HClO_4$-soluble, not adsorbed by Norite A); Triangles = Nucleotide-P ($HClO_4$-soluble, adsorbed on Norite). Open symbols: DNP. Filled symbols: 2,4-D.

although not entirely, controlled by the amount of undissociated acid present [4], further experiments compared 2,4-D with DNP at equal concentration of undissociated acid. For this purpose the pKa of 2,4-D was taken as 2.96 and that of DNP as 4.00, with the result that the total concentration of 2,4-D required was slightly more than 10 times that of DNP for comparable concentration of undissociated molecules.

The effect of these two compounds on P^{32} uptake into the inorganic phosphate fraction (acid soluble, not adsorbed by Norite), which of course includes neutral phosphate esters and other compounds as well, is shown in the upper two lines of Figure 1. It may be seen that over a range of undissociated acid concentrations from 10^{-8} to 10^{-4} *M* these materials exert little, and essentially the same effect on the inorganic phosphate fraction of Chlorella cells. As is shown in the lower two lines of Figure 1, 2,4-D and DNP in undissociated acid concentrations higher than 10^{-6} *M*, which corresponds to a total acid concentration of 3.5×10^{-4} *M* for 2,4-D and 3.3×10^{-5} *M* for DNP, cause a progressive diminution in the amount of P^{32} incorporated into nucleotides. The maximal decrease in the P^{32} of this fraction is about 100-fold at the highest undissociated acid concentration used. The curves for 2,4-D and DNP are similar. The apparent differences may arise from the fact that although the effect of weak acids is influenced primarily by the concentration of molecules in solution, it is possible that ions may also penetrate at a lower rate [5]. The greater population of ions in the 2,4-D solution could result in the greater effect found at low concentrations.

Comparison of 2,4-D and DNP effects on oxygen uptake

Changes in the rate of oxygen uptake by Chlorella due to 2,4-D and DNP and their effects on the respiratory quotient were studied with a Warburg respirometer. One ml of the concentrated cell suspension plus 1 ml of 0.02 *M* K-phosphate or 0.02 *M* K-citrate buffer at pH 5.5 was placed in each of two 15 ml flasks for each treatment. One flask of each pair contained 10 per cent KOH in the center well; the other contained water. Flasks were shaken in the dark at 25° C for a 20-minute equilibration, the system was closed and readings taken for 30 minutes before tipping 0.7 ml of 2,4-D or DNP dissolved in 0.01 *M* buffer into the flask from a side arm to give the desired final concentration. The rates between 30 and 60 minutes after tipping in the acids were used for calculation of RQ and comparison of effects on respiration.

The influence of 2,4-D and DNP in comparable undissociated acid concentration on oxygen uptake by Chlorella is shown in Figure 2. The effect of these materials is quite similar, with a small degree of stimulation found up to an undissociated acid concentration of

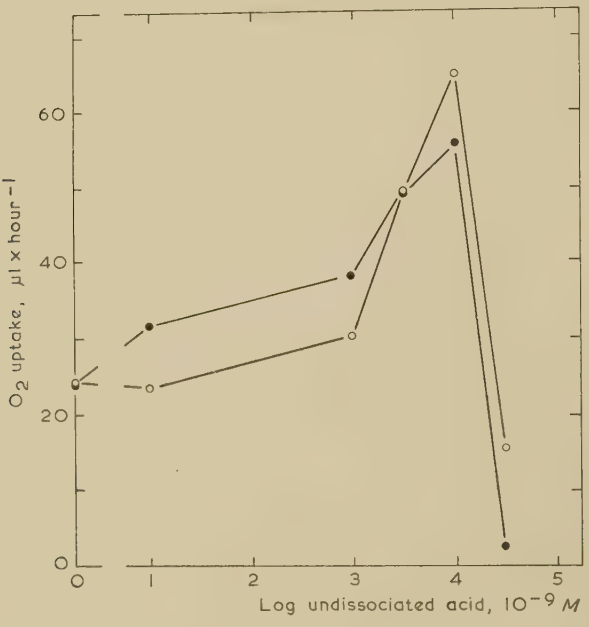

Fig. 2. Effects of 2,4-D and DNP in equal concentrations of undissociated acid on the respiratory rate of Chlorella. In 0.01 *M* K-phosphate buffer, pH 5.5. Measurements taken between 30 and 60 min after tipping in acids. Open symbols: DNP. Filled symbols: 2,4-D.

10^{-6} *M*, followed by a rapid increase to a maximal rate 2.5 to 3.0 times that of the untreated cells at 10^{-5} *M*, and a marked inhibition at higher concentrations. This figure shows results with endogenous oxygen uptake, but in the presence of citrate, relatively the same effect of the inhibitors is found although the overall rate is about three times the endogenous rate.

The respiratory quotient of the cells is altered in a pattern which is essentially the same for both compounds as is shown in Table 2, where the fall in RQ is followed by a rise at the highest concentration of both toxicants.

TABLE 2

Respiratory quotients of Chlorella treated with 2,4-D or DNP in equal concentrations of undissociated acid

Undissociated acid concentration — M	Respiratory quotient			
	Endogenous		Plus citrate	
	2,4-D	DNP	2,4-D	DNP
0	0.90	0.90	1.44	1.44
1×10^{-8}	1.15	1.20	1.42	1.44
1×10^{-6}	1.00	1.16	1.21	1.16
3.2×10^{-6}	0.90	0.97	0.93	0.96
1×10^{-5}	0.88	1.16	0.86	1.03
3.2×10^{-5}	3.00	1.85	2.65	1.64

DISCUSSION

The evidence offered here does not conclusively prove that 2,4-D and DNP affect phosphorylation in Chlorella in the same manner. The hypothesis that 2,4-D can uncouple phosphorylation in Chlorella as has been reported in other plant and animal preparations [1-4] is supported by the preferential inhibition of phosphate incorporation into ATP and ADP in the presence of 2,4-D and the coincidence of a decreased incorporation of phosphate into nucleotides with respiratory stimulation.

The question of whether 2,4-D is as effective as DNP in preventing phosphorylation can only be resolved by a kinetic analysis of the problem using labelled 2,4-D and DNP to relate the degree of inhibition to the amount of the acids which actually penetrate the cells. However, the manner in which equal concentrations of molecules of the two acids produce similar effects on phosphate incorporation, oxygen uptake and RQ suggests that they are almost equally active as uncoupling agents for Chlorella.

SUMMARY

The plant growth regulator 2,4-D inhibits the incorporation of $P^{32}O_4$ into ATP and ADP by Chlorella to a much greater extent than it does the accumulation of inorganic phosphate by the cells.

Comparison of 2,4-D effects on P^{32} incorporation into nucleotide-P with those of 2,4-dinitrophenol shows that when supplied as equal concentrations of undissociated acid molecules, they are nearly equally effective.

Both 2,4-D and DNP in concentrations which inhibit the incorporation of P^{32} into nucleotides stimulate the uptake of oxygen by Chlorella, supporting the suggestion that they are effectively uncoupling phosphorylation.

Received August 25, 1960

REFERENCES

1 Brody, T. M., Effect of certain plant-growth substances on oxidative phosphorylation in rat-liver mitochondria. Proc. Soc. Exptl. Biol. Med. **80**, 533–536 (1952).
2 Laties, G. G., The nature of the respiratory rise in slices of chicory root. Arch. Biochem. Biophys. **79**, 364–377 (1959).
3 Simon, E. W. and Beevers, H., The effect of pH on the biological activities of weak acids and bases. I. The most usual relationship between pH and activity. New Phytologist **51**, 163–190 (1952).
4 Switzer, C. M., Effects of herbicides and related chemicals on oxidation and phosphorylation by isolated soybean mitochondria. Plant Physiol. **32**, 42–44 (1957).
5 Wedding, R. T. and Erickson, L. C., The role of pH in the permeability of Chlorella to 2,4-D. Plant Physiol. **32**, 506–512 (1957).

Plant and Soil XIV, no. 3 May 1961

THE DECOMPOSITION OF ORGANIC MATTER IN A MANGROVE SWAMP SOIL

by P. R. HESSE

West African Rice Research Station, Rokupr, Sierra Leone

INTRODUCTION

The reclamation of coastal, Rhizophora mangrove swamps for the cultivation of rice in West Africa is becoming important. Owing to the tendency for Rhizophora to form fibrous muds and the propensity of such muds to become intensely acid on drying, the reclamation of these mangrove swamps has presented some difficulty (Dent [7], Tomlinson [14]).

The fundamental problem in reclaiming Rhizophora swamps arises from the facts that they have to be empoldered to exclude salt water and that their fibrous muds accumulate oxidisable sulphur. Empoldering the swamps results in the drying out of the topsoil during the dry season and hence to the production of acid conditions inimical to the growth of rice. This aspect of mangrove swamp reclamation has received considerable attention (Tomlinson [15], Hart [9]) and investigations still continue.

Another and hitherto unconsidered result of empoldering and subjecting the mangrove mud to seasonal drying will be a modification of the mode and rate of humus decomposition. It has long been known that drying a soil will produce a flush in the decomposition of its organic matter on re-wetting, but only recently since the investigations of Birch [1 2 3 4], have the practical implications of a definite wet-dry season been appreciated. In the case of mangrove swamp muds which have remained wet and periodically submerged for many years, the introduction of a regular wet dry season can be expected to have far-reaching consequences.

For the successful cultivation of rice the toxic substances pro-

duced when the mud is dried will either have to be made non-effective or removed. If proposed reclamation schemes take place the removal of chloride and if necessary, soluble acids, from the empoldered swamps will be due largely to leaching with rainwater.

This investigation therefore, deals with the decomposition of organic matter in the mud of a Rhizophora mangrove swamp and the effect upon that decomposition of empoldering the swamp. The further effect upon humus decomposition when the muds are leached with rain water has also been studied as have the additional influences of fertilising and liming.

MATERIALS AND METHODS

The experimental site was a coastal mangrove swamp at Wellington near Freetown in Sierra Leone. The swamp was tidally flooded twice a day and the soil consisted of a soft, silty deposit of from 3 to 10 cm thick overlying a compact, fibrous layer of black mud which extended to a depth of several feet.

Analyses

Sulphate was measured by Hesse's [10] modification of the method of Chesnin and Yien [6] and the same extract was used to determine phosphate; this was done using Truog's reagent and has been taken as an arbitrary measure of inorganic phosphorus. pH values were measured electrometrically using a glass electrode and ammonium and nitrate were measured by the distillation method using a potassium chloride extract made at pH 2.

Incubations were done in the usual manner and at 32°C and respirometric studies were made using the macro-respirometer designed by Birch and Friend [5].

The rain water falling direct to earth and that dripping from the mangrove leaves was collected and analysed by methods previously described (Hesse [11]).

The soil was sampled from the fibrous layer under a stand of *Rhizophora racemosa*.

EXPERIMENTS AND RESULTS

Experiment 1. *The effect upon rate of decomposition of organic matter when the mud is allowed to dry gradually*

The mud was allowed to dry slowly in the air with daily mixing so as to give samples with moisture contents varying from 5 per cent to field moisture and these samples, after crushing where

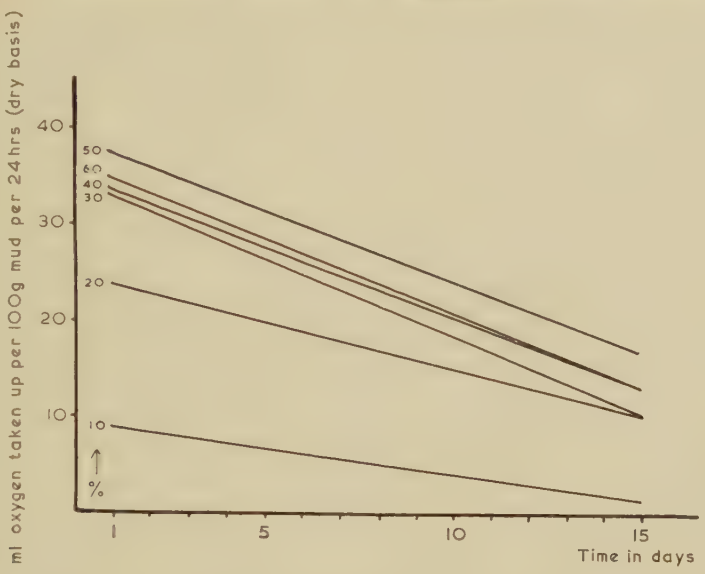

Fig. 1. Rate of oxygen uptake by fibrous mud as affected by moisture content.

necessary, were incubated in the respirometer for fifteen days. The rates of oxygen uptake at the various moisture contents are shown in Figure 1 from which it can be seen that the optimum moisture content for oxygen uptake was 50 per cent. The field moisture content at low tide, 60 per cent, permits almost as great a rate of oxidation however and in fact there is little difference at moisture contents of from 60 to 30 per cent.

Below 30 per cent moisture the rate of humus decomposition fell off, at 10 per cent the decomposition was seriously impeded and at moisture contents below 10 per cent there was no measurable decomposition. Thus the fibrous mud behaved very similarly to the forest soil, described by Birch [1] who found that less than 11 per cent moisture was insufficient for microbial activity and that the rate of decomposition was proportional to moisture contents of between 14 per cent and field capacity.

Experiment 2. *The decomposition of organic matter in dried mangrove mud and the effect upon this of treatments with lime and fertilisers*

This experiment was designed to examine the effect of empoldering upon humus decomposition which would occur during

the first few weeks of rain after a dry season in a cleared mangrove swamp. During this period the land would be lying fallow.

Measurements were made of changes in content of ammonia, nitrate, sulphate, and phosphate. The quantities of carbon oxidised and the rates of humus decomposition were measured and changes in pH value were followed.

Samples of mud as described in Table 1 were incubated in the respirometer for one month; fresh mud was also incubated as a means of comparison for assessing the effect of drying. The fresh mud was incubated at its field moisture content at low tide, that is 60 per cent, as being the most convenient, the most natural and, as shown in Experiment 1, almost optimum for humus decomposition. The pre-dried mud was re-wetted to the same consistency as the fresh mud; complete restoration of the original field moisture was impossible as drying the mud reduced its water-holding capacity. The dried muds were inoculated with 0.1 g of fresh mud before incubation.

Lime was added in quantities equivalent to a dressing of 14 tons calcium carbonate per acre 6″, the figure arrived at as being the minimum quantity necessary for ameliorating the excessively acid conditions. Phosphate was added as mono-basic calcium phosphate at 0.2 per cent and nitrogen as calcium nitrate in quantities sufficient to make the C/N ratio about 10 so as to avoid its immobilisation.

Duplicate samples of dried, fibrous mud were periodically removed from the respirometer, re-dried, re-moistened, and replaced after re-inoculating. This was to show the effect of repeated wet–dry cycles upon the rate of decomposition.

The experimental results are given in Table 1 and the rates of humus decomposition are shown graphically in Figure 2.

Aerobic incubation increased the acidity of the mud but this was prevented by liming. Phosphate maintained the pH values above 5 and nitrate had little effect. There was the expected increase in amounts of sulphate and this was greatest in the pre-dried mud. Liming the mud slightly increased the quantity of sulphur oxidised but both phosphate and nitrate decreased it.

On adding calcium phosphate to the dried mud approximately one fifth of it was immediately rendered unavailable to acid extraction. On incubation another three fifths were fixed and thus only about one fifth of the added phosphate was still extractable after one month's incubation.

Drying the mud increased its mineral nitrogen content and this was further increased with subsequent incubation. Although drying produced small amounts of nitrate, this disappeared during incu-

TABLE 1

Aerobic incubation of dried and re-wetted mangrove swamp muds with and without addition of fertilisers, for one month at 32°C
(Results expressed on oven-dry basis)

Determination of		Fresh mud	Dried and re-wetted mud			
			Alone	+ $CaCO_3$	+ $Ca(NO_3)_2$	+ $CaH_4(PO_4)_2$
pH	Initial	6.6	4.6	6.7	5.5	5.2
	Final	4.6	3.3	6.9	3.9	5.3
NH_4-N	Initial	10	25	25	25	25
ppm	Final	16	48	53	636	77
NO_3-N	Initial	1	10	10	7500	10
ppm	Final	2	0	0	7840	0
PO_4-P	Initial	18	9	9	9	375
ppm	Final	28	15	10	11	128
SO_4-S	Initial	1667	3685	3685	3685	3685
ppm	Final	4160	5350	5470	5250	5250
CO_2-C produced ppm		940	1630	1980	957	1002

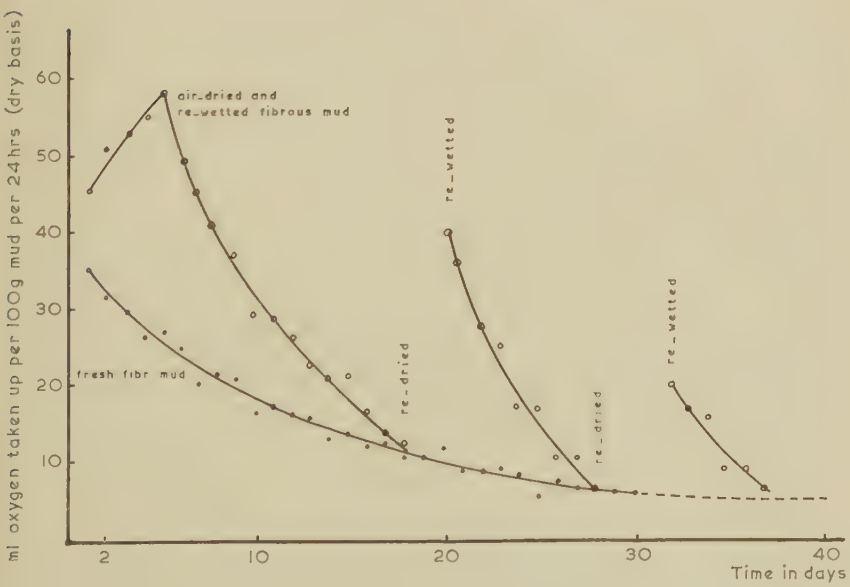

Fig. 2. The effect of repeated drying and wetting of fibrous mud upon its rate of oxygen uptake.

bation. Calcium carbonate and calcium phosphate on the whole increased the nitrogen ammonified in the dried mud. The effect of the very heavy dressing of nitrate was to increase greatly the amounts of organic nitrogen mineralised, the extra nitrogen appearing as both ammonia and nitrate.

The oxygen uptake of the mud was nearly all accounted for by carbon and sulphur oxidation. Thus in one month 429 ml of oxygen were used to oxidise 0.094 g of carbon and 0.2492 g of sulphur and the measured oxygen uptake converted to NTP was 438 ml.

Drying the mud resulted in a flush of decomposition which lasted for approximately eighteen days and caused an increase of 690 ppm carbon oxidised over that in the fresh mud. Once again the oxygen taken up (605 ml at NTP) was practically all accounted for by carbon (304 ml) and sulphur (270 ml) oxidation.

If drying the mud did not cause such an increase in its acidity which inhibits microbial activity, much bigger flushes of decomposition would occur and this is apparent if the results of incubation of dried mud with added lime are considered.

As shown in Figure 2, with successive dryings and wettings the magnitude of the flushes of decomposition fell off each time, an effect which has also been found with acid upland soils. Thus Birch [1] found that after only twelve successive dryings of an acid (pH 4.8) soil, a very small flush of decomposition occurred, only 9 per cent of the original carbon having been oxidised. With the dried mangrove soil which has a pH value of 3 or less, even smaller flushes of decomposition are to be expected.

The addition of phosphate and nitrate had the effect of slightly decreasing the rates of decomposition of the dried mud. Shaw [13] has recorded that inorganic P and N added to grass mats formed over acid pasture soils decreased the rate of decomposition of the organic matter unless lime was also present, when the rate was increased. It is thus feasible to assume that if the acidity of the mud was reduced by liming, then phosphate and nitrate would increase the rate of humus decomposition.

Experiment 3. *Decomposition of organic matter in dried mud when incubated under waterlogged and anaerobic conditions*

After several months rain has fallen, the empoldered swamp may, depending upon the drainage, become waterlogged or even perma-

nently under water when anaerobic conditions could prevail. This experiment therefore, was designed to examine the changes which would take place if such conditions should arise.

Samples of mud were treated as listed in Table 2 and incubated for one month in conical flasks. For the waterlogged incubations the muds were under one inch of standing water and for anaerobic incubation all air was displaced with nitrogen, the muds being under one inch of standing water as before. The results are given in Table 2.

Waterlogged and anaerobic conditions resulted in similar changes in the composition of the mud except that the more intense reducing conditions of the anaerobic incubation were reflected in certain analyses. Thus phosphorus was rendered more available than under waterlogged conditions and the quantities of ammonium nitrogen formed tended to be greater. pH values were raised in every case; that of the mud alone for example, which under aerobic conditions had a pH of about 3 (Table 1), attained a value of over 6 during its waterlogged and anaerobic incubations.

TABLE 2

Waterlogged and anaerobic incubation of dried and re-wetted mangrove swamp muds for one month at 32°C
(Results expressed on oven-dry basis)

Determination of		Mud alone	+$CaCO_3$	+$Ca(NO_3)_2$	+$CaH_4(PO_4)_2$
pH	Initial	4.6	6.5	5.5	5.2
	Final W	6.2	6.7	6.0	5.8
	Final A	6.3	6.9	6.1	6.2
NH_4 N ppm	Initial	25	25	25	25
	Final W	138	224	1400	196
	Final A	261	277	1440	286
NO_3-N ppm	Initial	10	10	7500	10
	Final W	0	0	6640	0
	Final A	0	0	6080	0
PO_4-P ppm	Initial	9	9	9	375
	Final W	5	5	3	150
	Final A	54	34	20	460
SO_4-S ppm	Initial	3685	3685	3685	3685
	Final W	1800 *	1920 *	3880	2280 *
	Final A	2200 *	2080 *	3280	2240 *

W = Waterlogged incubations
A = Anaerobic incubations
* = Presence of sulphide

The general decrease in acidity of the mud was reflected in a reduction of sulphate to sulphide. The presence of nitrate however, prevented such reduction due to its stabilising effect on the E_h of the system. Recently Vámos [17] has suggested the use of nitrate as a means of preventing the reduction of sulphate in paddy fields.

Lowering the C/N ratio by addition of nitrate caused increased mineralisation of organic nitrogen although a considerable amount of the added nitrate was remaining after one month's incubation.

Experiment 4. *The effect of leaching with rain-water upon decomposition of organic matter in mangrove swamp mud*

In Table 3 is given the analysis of the rainwater samples. The early rains in Sierra Leone are associated with violent electric storms and the rainwater during this period contains more mineral nitrogen than it does during the rest of the season (Hesse [12]), and it can be seen from the Table that in the particular area examined the early rains brought down over twice as much nitrogen as did the late rains.

TABLE 3

Analysis of rainwater falling onto a tidal mangrove swamp					
Type of rain		SO_4-S ppm	PO_4-P ppm	Mineral-N ppm	Cl ppm
Rain falling direct to earth	Early rains	0.7	0.01	0.18	5.0
	Late rains	0.6	0.14	0.08	3.6
Rain falling via Rhizophora leaves	Early rains	1.4	0.05	0.10	10.7
	Late rains	0.9	0.16	0.11	3.6

The early rains were collected during the first heavy downpour of the season and which occurred in April. The late rain was collected during October.

For the leaching experiment the mud samples were mixed with twice their weight (oven-dry basis) of pure sand and placed on a pad of glass wool in a wide-mouthed, cylindrical separating funnel. Liming was done by mixing calcium carbonate with the muds at a rate of 14 tons per acre 6″. Rainwater in measured quantities was then allowed to percolate slowly through the columns of mud until no more chloride was leached out. Attempts to leach out all the soluble sulphate which is more strongly absorbed than is chloride, were abandoned as being too time-consuming. However, as the analyses show (Table 4), the soluble sulphate contents were greatly reduced.

After leaching and allowing to drain overnight pH measurements were

made and then samples of the mud/sand mixtures were taken for analysis and duplicate samples were incubated in the respirometer for fifteen days. Corresponding unleached samples were incubated at the same time.

TABLE 4

The effect of leaching upon oxidation of organic matter in limed and unlimed mangrove swamp muds

Determination of		Fresh mud			Dried mud		
		Unleached	Leached with rainwater	Limed and leached + rain	Unleached	Leached with rainwater	Limed and leached + rain
Cl %		2.5	0	0	3.1	0	0
pH	Initial	6.6	6.8	8.1	4.6	5.9	7.9
	Final	4.6	3.2	7.7	3.9	4.8	7.5
NH_4-N ppm	Initial	7	0	1	20	4	6
	Final	20	10	8	42	30	31
NO_3-N ppm	Initial	4	1	1	14	2	1
	Final	0	0	0	0	0	0
PO_4-P ppm	Initial	20	4	82	15	11	66
	Final	23	11	39	13	29	17
SO_4-S ppm	Initial	1750	128	197	3800	274	266
	Final	3386	1460	460	6720	1026	665
CO_2-C mg/100 g		79	120	96	86	60	123

Results are expressed on the basis of oven-dry mud, unmixed with sand.
The "Final" results are those obtained after incubation for 15 days.

The results are given in Table 4 and show how leaching with and without addition of lime affects the composition of the muds both immediately and after incubation. Figure 3 shows how such treatments affect the rate of humus decomposition.

The immediate effect of leaching the fresh, unlimed mud was to raise slightly the pH value but not to the extent that could be expected on leaching a saline mud although possibly some unsaturation was caused. On incubation the pH of the leached mud dropped to a value even lower than that reached by the unleached mud.

The sulphate present after fifteen days incubation was independant of whether or not the mud had been leached, although presumably the leaching effect would appear in total- or organic-sulphur analyses.

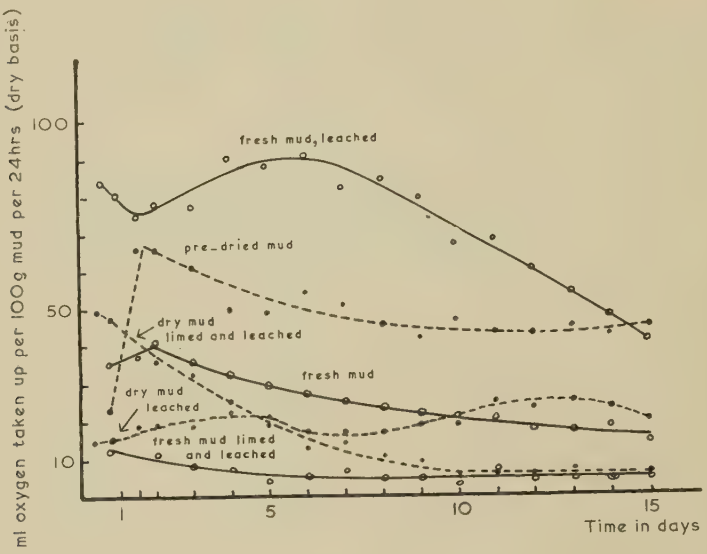

Fig. 3. Rates of oxygen uptake by fibrous mud (fresh and pre-dried) as affected by leaching with and without previous treatment with lime. The unbroken lines refer to the fresh muds and the broken lines to pre-dried muds.

As might be expected most of the acid-soluble phosphate was removed by leaching although its concentration was increased again on incubation. Similarly the inorganic nitrogen removed was subsequently re-formed.

Leaching the fresh mud led to an increase in carbon oxidation. As pointed out by Birch [2], salts will depress the solubility of organic matter and consequently after leaching more organic matter will become available for the Stage-I decomposition process.

Liming the fresh muds before leaching modified the leaching effect considerably. In the first place the presence of lime maintained the soil structure during leaching; when unlimed mud was leached the clay deflocculated and drainage was impeded. Liming naturally caused an increase in pH values which was still apparent after leaching and after incubation. The presence of lime resulted in less sulphate being removed during the leaching but on subsequent incubation very much less sulphate was present than in the unlimed or unleached muds. Wiklander *et al.* [18] have stated that the addition of lime, even in small quantities insufficient to neutralise the acid present, will increase sulphur oxidation, but

as found by Hart [9], the high pH resulting from heavy dressings of lime is unfavourable for the oxidation of sulphur. Such an effect would obtain here where the dressing of lime was very heavy. Liming had little effect upon nitrogen oxidation but resulted in increased quantities of calcium-bound phosphate. This available phosphate in spite of high pH values being maintained during incubation, fell to the original low concentration after fifteen days.

The rate of oxygen uptake after leaching was depressed by lime and this can in part be explained by the inhibitory effect of lime upon the sulphur-oxidising organisms. For example in the leached mud 140 ml of oxygen were utilised for sulphur oxidation whereas in the limed and leached mud only 28 ml of oxygen were involved in the oxidation of sulphur. Another reason for the high oxygen uptake by the leached mud has recently been shown by Hart (priv. comm.) to be due to oxidation of iron and this too, is suppressed by addition of lime. Thus the reduced oxygen uptake of the fibrous mud after liming does not necessarily reflect a reduced rate of organic matter disintegration.

If the mud was dried before leaching there was a drop in initial pH value and a corresponding increase in soluble sulphate. On leaching more sulphate was removed than from the undried mud and a rise in pH occurred. Liming the dried mud before leaching caused similar pH and sulphate changes as did liming the fresh mud.

Incubating the re-wetted, pre-dried mud as already shown in Experiment 2 resulted in an enhanced carbon and nitrogen oxidation, but leaching the dried mud before incubation removed the easily soluble organic matter and consequent C and N oxidation was less than in the unleached muds despite the removal of salts. It would appear that to obtain maximum humus decomposition in unlimed muds it would be necessary to leach out excess salts before drying. The effect of lime added to dry mud before leaching was to enhance the subsequent carbon and nitrogen mineralisation.

DISCUSSION

It is suggested that during reclamation coastal mangrove swamps should first be cleared during a dry season, the muds remaining under tidal conditions. Just before the wet season sets in the

swamp should be empoldered to exclude tidal water and some form of drainage system would be applied. The land would then lie fallow throughout the ensuing rains and the next dry season.

After empoldering and as the rainy season progressed, the mud now excluded from tidal effects and from the supply of fresh organic matter would be leached and the probable results of this have been shown by Experiment 4. The pH values would be slightly raised but the leaching would induce increased sulphur oxidation and if for any reason the leaching process ceased the mud would become more acid than before. An important practical point is that unless the mud was limed its physical structure would deteriorate under leaching. However, as too alkaline conditions can inhibit sulphur oxidation, whereas a moderate increase in pH would encourage production of sulphates, the amount of lime added would have to be carefully controlled in order that the leaching of sulphates is carried out with maximum efficiency.

A loss of inorganic nitrogen and phosphorus would occur and owing to removal of salts, the rate of humus decomposition would increase; the products of this may well be washed out of the soil.

As the mud becomes drier during the next dry season the rate of humus decomposition would become progressively slower as shown by Experiment 1, until during the latter part of the dry season no significant decomposition would take place.

Decomposition of humus would become really significant during the first week of the following rainy season when the re-wetting of the dry soil would initiate a flush of decomposition with consequent liberation of inorganic nitrogen. The intense acidity of the mud at this stage however, would prevent nitrogen mineralisation being as high as if the mud had been previously limed. In fact most of the increase in oxygen uptake would be due to sulphur, iron and carbon oxidation although this would lead to a reduction in the C/N ratio. It has further been shown that if the mud was fertilised with phosphate this would maintain a higher pH status, probably due to immobilisation of aluminium ions. Thus phosphate fertilisation would, like lime, increase nitrogen mineralisation.

Also, as shown by Experiment 4, leaching out of salts would have permitted maximum humus decomposition and thus the periods when conservation of humus is desirable because the land is lying fallow, that is the dry season and its preceding wet season,

would be those in which least oxidation of organic matter occurs.

As the rainy season progresses the rate of humus decomposition will again fall off until a steady, slow rate is reached. Although some leaching would be taking place anaerobic conditions might begin to appear although this would depend upon the drainage system. If anaerobic conditions take effect the changes which can be expected to occur in the mud have been indicated by Experiment 3. The pH status of the mud would rise partly due to loss of soluble acids and partly to the reducing conditions. The pH of the fibrous material itself might drop a little due to anaerobic formation of organic acids but these in time would either decompose further or be leached away. Some sulphate would be reconverted to sulphide but this could be prevented by the addition of nitrate and thus one could possibly continue the removal of sulphates by leaching.

During the early rains after a dry season the fibrous material would decompose at its highest rate and it seems that if the decomposition of the fibres is desired, then the land should be treated with phosphate, lime, and inorganic nitrogen before drying.

It is noticeable that as rice is usually transplanted some time after the commencement of the rains, the plants will lose the benefit of the inorganic nitrogen released by the drying effect as well as that brought down by the rain. As the rice plant benefits from application of nitrogen at about the time of transplanting it may be desirable to catch the nitrogen flush by some system of premature transplanting with temporary irrigation.

It should not be forgotten that leaching or drying and leaching the mangrove swamp muds may produce effects other than those yet considered. For example, leaching such large quantities of sulphate could lead to a trace-element deficiency such as that of copper, zinc, or manganese. Again, as pointed out by Uchiyama and Onikura [16], on drying a paddy soil it will weather and subsequent leaching will remove silica thus leading to an accumulation of alumina. On the other hand, with soils containing slowly decomposing organic matter and with high C/N ratios, aluminium and iron will be leached in preference to bases (Duchafour [8]).

Finally it must be emphasized that empoldering the swamps would *inter alia*, preclude the regular deposition of alluvium. Although this aspect of swamp reclamation has not been considered

in this study, its importance as a contributing factor to the fertility of the land should not be overlooked.

SUMMARY

The decomposition of organic matter in the fibrous mud of a *Rhizophora racemosa* mangrove swamp has been investigated. Emphasis has been placed upon the effects of empoldering the swamps which operation leads to the seasonal drying of the topsoil. The added effects of leaching, fertilising, and liming upon the decomposition process have been examined.

Decomposition of humus is likely to be most significant during the early weeks of rain following a dry season. However, the mineralisation of nitrogen was greatly depressed by the intense acidity produced and a large proportion of the oxygen uptake by the mud was accounted for by sulphur oxidation. Both lime and phosphate maintained higher pH values in the mud and increased nitrogen mineralisation.

Leaching the muds from newly cleared mangrove swamps slightly raised the pH values but induced increased sulphur oxidation. Thus if the leaching of the mud was interrupted then it tended to become even more acid.

The physical structure of the mud deteriorated with leaching unless lime had been added. Too much lime however, inhibited sulphur oxidation and reduced the efficiency of sulphate removal by leaching. Owing to removal of salts, leaching increased the rate of humus decomposition and the maximum decomposition was obtained by drying and re-wetting muds which had been leached.

During the latter part of the rainy season when waterlogged conditions could occur, the reduction of sulphate to sulphide can be prevented by nitrate additions thus enabling more sulphate to be removed by leaching.

It has been concluded that the decomposition of the fibrous material found in muds under Rhizophora can be accelerated by treatment with phosphate, lime, and inorganic nitrogen, the lime at least being added before the mud is permitted to dry.

The practical implications of leaching and empoldering mangrove swamp muds are discussed and a suggestion has been made for utilising in rice cultivation the nitrogen flush which occurrs at the beginning of a rainy season.

ACKNOWLEDGEMENT

This paper is published with the permission of the Director of Agriculture, Sierra Leone.

Received July 6, 1960. Revised draft January 19, 1961

REFERENCES

1 Birch, H. F., The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil **10**, 9 (1958).
2 Birch, H. F., Simultaneous decomposition processes in soils. Nature **183**, 415 (1959).
3 Birch, H. F., Further observations on humus decomposition and nitrification. Plant and Soil **11**, 262 (1959).
4 Birch, H. F., Nitrification in soils after different periods of dryness. Plant and Soil **12**, 81 (1960).
5 Birch, H. F. and Friend, M. T., Humus decomposition in East African Soils. Nature **178**, 500 (1956).
6 Chesnin, L. and Yien, C. H., Turbidimetric determination of available sulphates. Soil Sci. Soc. Am. Proc. **15**, 149 (1950).
7 Dent, J. M., Some soil problems in empoldered rice lands in Sierra Leone. Empire J. Exp. Agr. **15**, 206 (1947).
8 Duchaufour, P., Sur le rôle de la matière organique dans les phénomènes de lessivage et de podsolization. Compt. Rend. **245**, 1154 (1957).
9 Hart, M. G. R., Sulphur oxidation in tidal mangrove soils of Sierra Leone. Plant and Soil **11**, 215 (1959).
10 Hesse, P. R., The effect of colloidal organic matter on the precipitation of barium sulphate and a modified method for determining soluble sulphates in soils. Analyst **82**, 710 (1957).
11 Hesse, P. R., Sulphur and nitrogen changes in forest soils of East Africa. Plant and Soil **9**, 86 (1957).
12 Hesse, P. R., Ann. Rept. of the West African Rice Research Station, 1959.
13 Shaw, K., Decomposition of plant residues in grassland soils. Rep. Rothamsted Exptl. Sta. **1957**, p. 62 (1958).
14 Tomlinson, T. E., Relationship between mangrove vegetation, soil texture and reaction of surface soil after empoldering saline swamps in Sierra Leone. Trop. Agr. Trinidad **34**, 41 (1957).
15 Tomlinson, T. E., Changes in a sulphide-containing mangrove soil on drying and their effect on the suitability of the soil for the growth of rice. Empire J. Exptl. Agr. **25**, 108 (1957).
16 Uchiyama, N. and Onikura, Y., Investigations on clay formation in paddy soils. J. Sci. Soil Tokyo **25**, 291 (1955).
17 Vámos, R., Inhibition of sulphate reduction in paddy soil. Nature **182**, 1688 (1958).

Plant and Soil XIV, no. 3 May 1961

ÜBER DIE PHOSPHATIONENAUFNAHME DURCH PFLANZEN

I. PHOSPHATIONENAUFNAHME DURCH HAFER ALS FUNKTION DER PHOSPHATKONZENTRATIONEN IN NÄHRLÖSUNG UND PFLANZE

von F. SCHEFFER *, B. ULRICH und H. J. KAUFMANN

Agrikulturchemisches und Bodenkundliches Institut der Universität Göttingen

EINLEITUNG

Zahlreiche Beobachtungen haben gezeigt, daß die Pflanze nicht nur in der Lage ist, Nährstoffe in Form von Ionen aus einer Lösung aufzunehmen, sondern unter gewissen Umständen auch wieder abzugeben. Allgemein bekannte Fälle von Nährstoffabgaben finden wir bei reifenden Pflanzen und bei Neubauer-Untersuchungen mit Nährstoff-fixierenden Böden.

Nähere Betrachtungen zeigen, daß sich diese entgegengesetzten Nährstoffströme (Aufnahme – Abgabe) zwanglos erklären lassen, wenn man annimmt, das System Pflanze-Nährlösung sei ständig bestrebt, Gleichgewichte einzustellen: Durch das Wachstum der Pflanze werden diese Gleichgewichte immer wieder in einer Richtung gestört, was einen Nährstoffstrom in die Pflanze zur Folge hat. Kommt es jedoch zur Zeit der Reife in der Pflanze zu Abbauprozessen, so kann ein Ungleichgewicht in anderer Richtung entstehen und es kommt zu einer Nährstoffabgabe. Ähnliches gilt für die Neubauerversuche. Gut mit Nährstoffen versorgte Böden stellen in der Bodenlösung genug Ionen für eine Aufnahme durch die Pflanze bereit; bei den mit Sand gefüllten Kontrollgefäßen geben die Pflanzen geringe Mengen von ihrem Nährstoffvorrat aus dem Samen an die Lösung ab, bis zwischen Pflanze und Lösung das Gleichgewicht hergestellt ist; anders verhält es sich bei fixierenden Böden, hier werden die von der Pflanze abgegebenen Ionen von der

* Göttingen, Nikolausberger Weg 7.

festen Bodenphase der Lösung entzogen, so daß die Pflanze zur Einstellung des Gleichgewichtes laufend weiter Ionen abgeben muß. Dieser letzte Fall führt dann zu den bekannten negativen Werten im Neubauer-Versuch.

VERSUCHSDURCHFÜHRUNG

Zur Prüfung dieser Vorstellungen wurden eine Reihe von Versuchen angestellt, die im folgenden besprochen werden sollen. Als Versuchsmaterial dienten dabei drei-Wochen alte Haferpflanzen (Peragold Hochzucht), die mehrfach selektiert und dann zu 20 Pflanzen zu einer Versuchsgruppe zusammengefaßt wurden, um den individuellen Fehler klein zu halten. Anzucht und Versuche erfolgten auf einer Nährlösung, die zur besseren Vergleichbarkeit mit der Bodenlösung im Gegensatz zu sonst gewöhnlich verwendeten Nährlösungen nur eine Gesamtsalz-Konzentration von 1,0 bis 1,2‰ aufwies. Die Lösung war folgendermaßen zusammengesetzt (Angaben pro Liter Nährlösung):

KNO_3	40 mg	$(NH_4)_2SO_4$	15 mg
$Ca(NO_3)_2 \cdot 4H_2O$	60 mg	Fe (als Chelat)	0,3 mg
$MgSO_4 \cdot 7H_2O$	12,2 mg	Hoagland A–Z-Lösung	0,1 ml

Das entspricht folgenden Konzentrationen an Kernnährstoffen:

N	16,0 ppm	Ca	10,2 ppm
K	15,5 ppm	Mg	1,2 ppm
	Fe	0,3 ppm	

Die Nährstoffbewegung wurde am Beispiel des Phosphations untersucht. Zu diesem Zweck wurde der Phosphor der Nährlösung als KH_2PO_4 in Konzentrationen von 0 bis 5 ppm P zugesetzt. Zur Anzucht wurden 4-Liter-Töpfe mit 20 Pflanzen (= 1 Versuchsglied) verwendet, in denen die Lösung anfangs täglich, bei älteren Pflanzen zweimal in 24 Stunden gewechselt wurde. Bei jedem Wechsel wurde der P-Gehalt der alten Lösung und damit die P-Aufnahme durch die Pflanzen bestimmt.

ERGEBNISSE

Versuche zur Phosphationenaufnahme

Bereits bei der Anzucht der Pflanzen, die vom 6. bis zum 20. Tag bei verschiedenen Phosphatkonzentrationen erfolgte, ließen sich die ersten Hinweise auf die zu untersuchenden Fragen finden. Als Beispiel mag hier eine Versuchsreihe dienen, deren Phosphoraufnahme in Abb. 1 graphisch dargestellt ist. Mit den hier gewählten Phosphatkonzentrationen von 0; 0,2; 1,0 und 5,0 ppm P werden die verschiedenen Möglichkeiten, die eingangs abgeleitet

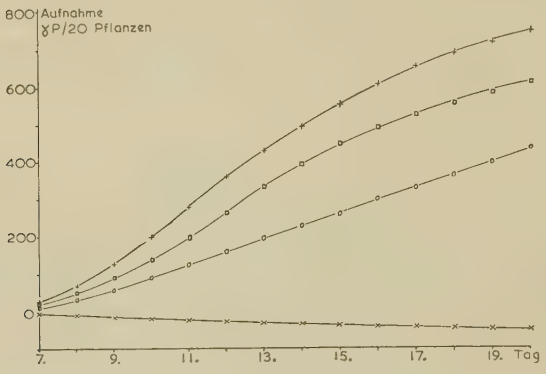

Abb. 1. Phosphoraufnahme durch 20 Haferpflanzen aus Nährlösungen mit verschiedenen P-Konzentrationen.
× = 0 ppm P □ = 1 ppm P
○ = 0,2 ppm P ÷ = 5 ppm P.

wurden, erfaßt: Bei Phosphor-freier Nährlösung haben wir eine laufende P-Abgabe der Pflanzen an die Lösung. Da sich alle Versuchsglieder während der ersten Woche der Anzucht auf einer P-freien Nährlösung befanden, kann es sich hier nur um Samenphosphorsäure handeln, die an die Lösung abgegeben wird. Daß die täglich abgegebene Menge langsam geringer wird, ist mit der durch die fortlaufende Abgabe und das Wachstum der Pflanzen ständig fallenden P-Konzentration in den Pflanzen zu erklären. Die nach Abschluß des Versuches anhand der Analysendaten aufgestellte P-Bilanz bestätigt diese Vorstellung (Tab. 1). Für die drei anderen Konzentrationen ergibt sich der für solche Versuche typische S-förmige Kurvenverlauf. Er entsteht durch eine anfängliche Anpassung der Pflanzen an das veränderte Milieu, eine anschließende Nachholphase, in der die Pflanzen ein neues Gleichgewicht mit der Lösung anstreben, und schließlich eine annähernd linear verlaufende dem täglichen Zuwachs entsprechende Aufnahme. Während dieser Verlauf bei 1 und 5 ppm P sehr ausgeprägt ist, ist bei 0,2 ppm kein Nachholbedarf zu verzeichnen, da hier der P-Gehalt der Sämlinge der Konzentration in der Lösung entspricht. Es wird hier also nach der Anpassungsphase gleich nur noch entsprechend dem täglichen Zuwachs aufgenommen.

In Abb. 2 wurde für die gleiche Versuchsreihe der in 14 Tagen von 20 Pflanzen aufgenommene Phosphor und die gebildete Trocken-

TABELLE 1

Phosphorbilanz einer Versuchsreihe					
Topf Nr.	ppm P in der Lösung	mg P im Pflanzen-material	mg P in 20 Samen	demnach aufgenom. P in mg	aufgen. P in mg nach Analyse der Lösungen
1	0	2,31	2,80	−0,49	−0,52
2	0,2	6,80	2,80	4,00	4,26
3	1,0	8,93	2,80	6,13	6,00
4	5,0	10,44	2,80	7,64	7,37

substanz als Funktion der P-Konzentration in der Nährlösung aufgetragen. Aus dieser Darstellung ist zu sehen, daß bereits eine Konzentration von 0,2 ppm P unter den gewählten Verhältnissen für ein normales Wachstum ausreicht. Dies deckt sich gut mit der Erkenntnis, daß unsere Ackerböden selten mehr als 1 ppm P in Lösung enthalten [2 7]. Da die Lösung trotz des häufigen Wechselns durch die Aufnahme immer wieder bis auf etwa 50 Prozent des Ausgangswertes gesenkt wurde, ist anzunehmen, daß bei genügend rascher Ergänzung des entzogenen P die P-Konzentration der Lösung noch niedriger liegen kann, ohne daß das Wachstum der Pflanzen leidet.

Während eine Erhöhung der P-Konzentration in der Lösung auf die Trockensubstanzbildung keinen Einfluß mehr hat, steigt der P-Gehalt der Pflanzen zunächst noch weiter an, hat aber – immer auf die gewählten Verhältnisse bezogen – bei etwa 5 mg/l auch seinen Sättigungswert erreicht. Bei P-Konzentrationen über 0,2 ppm in der Lösung setzt also bereits der Luxuskonsum an Phosphor ein.

Als nächstes galt es nun zu prüfen, inwieweit Aufnahme bzw. Abgabe der Nährstoffe von den absoluten Gehalten in Lösung und

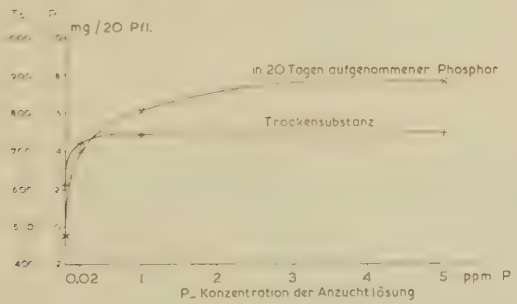

Abb. 2. Trockensubstanz (+) und aufgenommener Phosphor (×) von 20 drei Wochen alten Haferpflanzen nach Anzucht auf verschiedenen P-Konzentrationen.

Pflanze oder von dem gegenseitigen Verhältnis der Gehalte abhängig sind. Zu diesem Zweck wurden auf die oben beschriebene Weise angezogene Pflanzen auf ihre Aufnahme bzw. Abgabe bei verschiedenen Konzentrationen untersucht, indem sie für jeweils 20 Min. auf 300 ml Lösung mit verschiedenen P-Gehalten gesetzt wurden. Anschließend wurden dann die eingetretenen Änderungen in den P-Konzentrationen der Lösungen bestimmt und so der jeweilige Umfang der P-Aufnahme oder -Abgabe festgestellt. Abb. 3 zeigt die graphische Darstellung eines derartigen Versuches mit 4 Versuchsgliedern à 20 Pflanzen, die auf Lösungen zwischen 0 und 5 ppm P angezogen wurden und deren Aufnahme jeweils aus Lösungen zwischen 0 und 2 ppm P bestimmt wurde. Dabei wurden wegen des weiten Bereiches die P-Konzentrationen der Versuchslösungen logarithmisch aufgetragen.

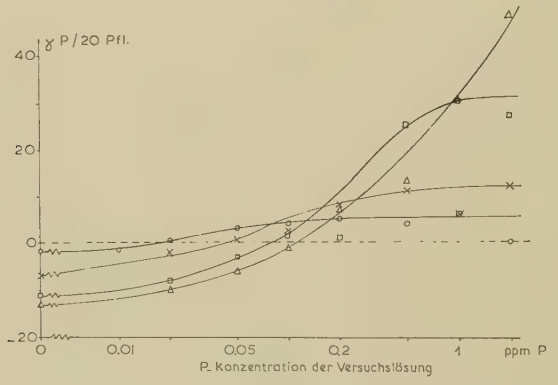

Abb. 3. Phosphoraufnahme bzw. -Abgabe durch 20 drei Wochen alte Haferpflanzen in 20 Min. auf 300 ml Nährlösung mit steigenden P-Gehalten.
○ = Anzucht auf 0 ppm □ = Anzucht auf 1 ppm
× = „ „ 0,2 ppm △ = „ „ 5 ppm.

Das starke Streuen der Ergebnisse bei den höchsten Konzentrationen der Versuchslösungen, das meßtechnisch bedingt war, läßt den Verlauf der Kurven in diesem Bereich nicht immer ganz klar erkennen; trotzdem kann man aber die wesentlichen Ergebnisse dieses Versuches gut ablesen: Einmal zeigt sich, daß es tatsächlich sowohl vom P-Gehalt der Pflanze als auch von dem der Lösung abhängt, wann wir Aufnahme oder Abgabe haben, denn der Punkt, an dem wir weder Aufnahme noch Abgabe finden, Pflanze und Lösung

sich also im Gleichgewicht befinden, wird mit steigender Anzuchtkonzentration und damit steigendem P-Gehalt der Versuchsgruppe zu immer höheren P-Konzentrationen in der Lösung verschoben.

Wie Abb. 4 und 5 zeigen, ist allerdings weder die Beziehung zwischen P-Konzentrationen in Anzucht- und Versuchslösungen noch die zwischen P-Gehalt der Pflanzen und Konzentration der Versuchslösung konstant.

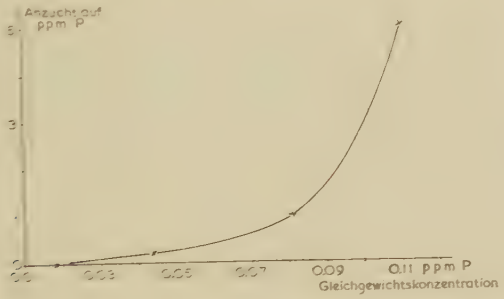

Abb. 4. Abhängigkeit der Gleichgewichtskonzentration für Haferpflanzen von dem P-Gehalt der Anzuchtlösungen.

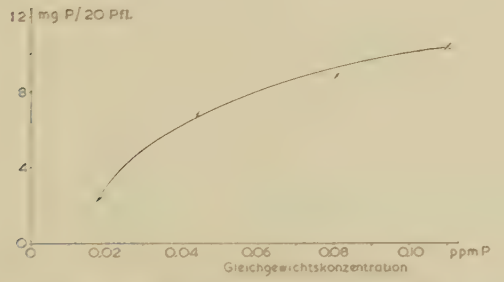

Abb. 5. Abhängigkeit von Gleichgewicht-konzentration und P-Gehalt für Haferpflanzen.

Beide Kurven zeigen vielmehr eine Krümmung etwa in dem Bereich, in dem nach den oben getroffenen Feststellungen der Luxuskonsum an P einsetzt. Da mit Einsetzen des Luxuskonsums andere P-Fraktionen stärker aufgefüllt werden als vorher, und wir von den verschiedenen P-Fraktionen einen unterschiedlichen Einfluß auf die Gleichgewicht-Einstellung erwarten müssen, wird diese Kurvenkrümmung verständlich.

Wenn bei der Kurve in Abb. 4 gerade der erste Teil der flachste ist, so bedeutet das, daß die auf P-armen Lösungen angezogenen Pflanzen nicht nur schlechter mit P versorgt sind, sondern außerdem auch noch auf Schwankungen in der Konzentration der Lösung viel stärker reagieren, indem sie relativ früher den Punkt erreichen, an dem keine Aufnahme mehr möglich ist, als das bei besser versorgten Pflanzen der Fall ist.

Das Abflachen der Kurve in Abb. 5 mit steigendem P-Gehalt der Pflanzen bedeutet, daß jede zusätzlich aufgenommene Einheit an P den Gleichgewichtswert der Lösung schneller erhöht, also in mobilerer Form in der Pflanze vorliegt und leichter wieder abgegeben wird. Das wurde ja schon oben mit dem unterschiedlichen Auffüllen der einzelnen P-Fraktionen in der Pflanze in Abhängigkeit von dem P-Angebot erklärt.

Abb. 3 veranschaulicht weiter, daß die Phosphor-Abgabe bei einer bestimmten Außenkonzentration desto stärker ist, je besser die Pflanzen mit P versorgt sind. Das entspricht den Vorstellungen über die Grundlagen der Aufnahme bzw. Abgabe, nach denen mit zunehmender Abweichung vom Gleichgewicht zwischen den Phasen das Bestreben auf Ausgleich stärker werden muß. Dem widerspricht andererseits die ebenfalls aus der Abbildung zu entnehmende Tatsache, daß bei höheren Außenkonzentrationen phosphatreiche Pflanzen eine stärkere Aufnahme haben als ärmer angezogene. Dies ist wohl damit zu erklären, daß die Pflanzen sich während der Anzucht in ihrem Stoffwechsel einer gewissen Außenkonzentration angepaßt haben und einige Zeit zur Umstellung benötigen. Diese Vorstellung wird durch ähnliche Verhältnisse im Wasserhaushalt der Pflanzen gestützt.

Es erhebt sich nun die Frage, ob die auf diese Weise erfaßten Größen wirklich die Gesamtaufnahme bzw. -abgabe repräsentieren oder ob die verwendeten Meßmethoden nur Nettobeträge erfaßt haben; denn eine weitverbreitete Anschauung ist heute, daß zumindest die erste Phase der Ionenaufnahme durch die Pflanzenwurzel auf Diffusion beruht [3 4], also ein ametabolischer und reversibler Prozeß ist. Die von Seiten der Pflanze an diesem Prozeß beteiligte austauschbare Ionenmenge wird in der angelsächsischen Literatur als A.F.S. (*apparent free space*) bezeichnet [1]. Es dürfte sich dabei in erster Linie um die Ionen handeln, die sich im Zellwandvolumen des Rindenparenchyms und der Epidermis befinden.

Wenn diese Ionen aber ständig mit denen in der freien Lösung im Austausch stehen, dann ist die mit den konventionellen Methoden meßbare Aufnahme bzw. Abgabe nur ein Nettobetrag, der die Differenz zwischen der nebeneinander verlaufenden Aufnahme und Abgabe darstellt. Der ganze Prozeß der Nährstoffaufnahme wäre also viel dynamischer als man bisher anzunehmen pflegte.

Isotopenaustausch-Versuche

Eine Bestätigung dieser Vorstellung läßt sich durch Einsatz von Isotopen gewinnen. Befindet sich ein Mehrphasensystem im chemischen Gleichgewicht, so kann man einen Ionenaustausch zwischen den Phasen auch für diesen Zustand nachweisen, indem man zu einer Phase ein Radioisotop des einen Reaktionspartners in so geringen Mengen zusetzt, daß das chemische Gleichgewicht praktisch nicht gestört wird. Da beim Ionenaustausch zwischen den einzelnen Isotopen nicht unterschieden wird, werden die Isotopen nach und nach auch im chemischen Gleichgewichtszustand über alle am Austausch beteiligten Phasen gleichmäßig verteilt. Es ist also durch diesen Isotopenaustausch möglich, den Doppelpfeil, mit dem ein kinetisches Gleichgewicht in statistischer Verteilung dargestellt wird, zu realisieren. Dementsprechend müßte sich auch ein etwa vorhandener isotoper Ionenaustausch bei der Nährstoffaufnahme mit Hilfe geeigneter Isotopen nachweisen lassen.

Das Studium von Isotopen-Austauschvorgängen stößt bei der Nährstoffaufnahme allerdings auf einige Hindernisse. Insbesondere ist es kaum möglich, die Pflanze für längere Zeit im Phosphorgleichgewicht mit der Nährlösung zu halten, da durch das Wachstum dieses Gleichgewicht immer wieder gestört wird, Austausch und Aufnahmevorgänge also nebeneinander ablaufen.

Versuche zum gleichen Fragenkomplex wurden bereits von Michael und Mitarbeitern [5] angestellt. Sie zogen die Pflanzen auf radioaktiven Lösungen an und setzten sie zu den Versuchen auf inaktive Lösungen. Die Zunahme der Aktivität in der Lösung war damit ein direktes Maß für den Austausch. Neben einigen Vorteilen hat diese Methode jedoch einen Nachteil: Sie bringt in Form der Aufzuchtlösungen große Mengen radioaktiver Abfälle, besonders dann, wenn die Lösungen wegen geringer Nährstoff-Konzentrationen oft gewechselt werden müssen. In den hier vorgelegten Versuchen wurde daher der umgekehrte Weg beschritten: Die Pflanzen wurden in der oben beschriebenen Weise auf inaktiven Lösungen angezogen und die Versuche dann mit den drei Wochen alten Pflanzen auf Töpfen mit 3750 ml markierter Lösung durchgeführt. Diese Versuche liefen etwa 10 Stunden. In dieser Zeit wurden laufend die Änderungen in der Phosphorkonzentration

der Lösung und ihrer Aktivität gemessen. Nach dem Versuch wurden die Pflanzen getrennt nach Sproß und Wurzel auf P^{31} und P^{32} analysiert.

Da bei Isotopenversuchen mit lebendem Material die Gefahr von Strahlenschäden am Versuchsmaterial besteht, mußten in dieser Hinsicht Vorsichtsmaßnahmen getroffen werden. Dies geschah einmal durch die geringen Aktivitäten (einige μc/l Nährlösung) und zum anderen durch die kurze Kontaminationszeit von wenigen Stunden. Einen sicheren Beweis, daß die Versuchsergebnisse nicht durch Strahlenschäden beeinflußt sind, erhält man, wenn man den gleichen Versuch mit verschiedenen Aktivitäten durchführt [6]. Von diesem Verfahren wurde bei den vorliegenden Versuchen Gebrauch gemacht. Es konnten – wie erwartet – keine Einflüsse der Strahlung auf die Ergebnisse nachgewiesen werden.

Die Abb. 6 und 7 zeigen eine Reihe derartiger Versuche, bei denen folgende Bedingungen gewählt wurden:

1. P-Konzentration in der Versuchslösung gleich der in der Anzuchtlösung,
2. P-Konzentration in der Versuchslösung höher als in der Anzuchtlösung,
3. P-Konzentration in der Versuchslösung geringer als in der Anzuchtlösung.

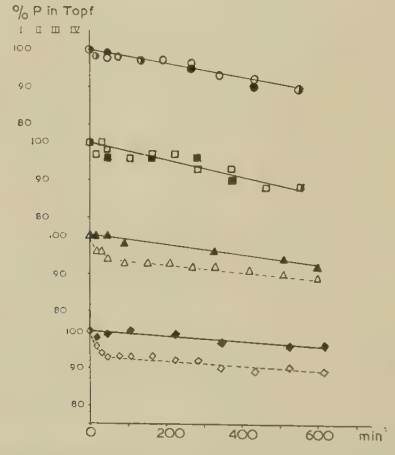

Abb. 6. Änderung des Gehaltes an P^{31} und P^{32} in 3750 ml Nährlösung durch Aufnahme bzw. Abgabe durch 20 drei Wochen alte Haferpflanzen.
P^{31} = ausgefüllte Zeichen, ausgezogene Linien
P^{32} = offene „ , unterbrochene „
○ = Anzucht auf 0 ppm, Versuch auf 1 ppm P
□ = „ „ 0,2 ppm, „ „ 1 ppm P
△ = „ „ 1 ppm, „ „ 1 ppm P
◇ = „ „ 5 ppm, „ „ 5 ppm P.

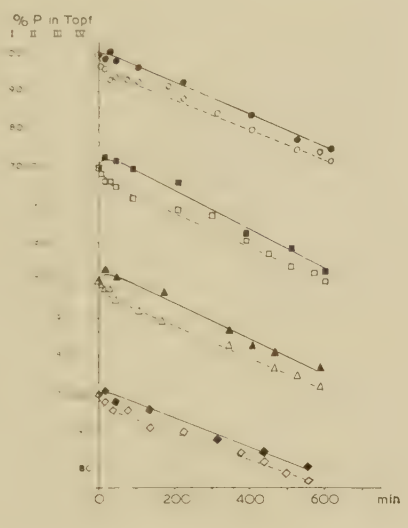

Abb. 7. Änderung des Gehaltes an P^{31} und P^{32} in 3750 ml Nährlösung durch Aufnahme bzw. Abgabe durch 20 drei Wochen alte Haferpflanzen.
P^{31} = ausgefüllte Zeichen, ausgezogene Linien
P^{32} = offene „ , unterbrochene „
○ = Anzucht auf 1 ppm, Versuch auf 0,2 ppm P
□ = „ „ 1 ppm, „ „ 0,2 ppm P
△ = „ „ 5 ppm, „ „ 0,2 ppm P
◇ = „ „ 5 ppm, „ „ 0,2 ppm P.

In den Fällen, in denen die Konzentrationen bei Anzucht und Versuch gleich waren (Abb. 6, die beiden unteren Kurven), kommt der Isotopenaustausch-Effekt sehr gut zum Ausdruck. Während der Gehalt an P^{31} in der Versuchszeit linear abfällt, ist die Abnahme an P^{32} zunächst sehr viel stärker, später verlaufen beide annähernd paralell zueinander. Das bedeutet, daß hier P^{32} durch den Austauschprozeß zunächst gegen P^{31} in die Pflanze gelangt, bis das Radioisotop über beide Phasen gleichmäßig verteilt ist. Anschließend werden beide Isotope in konstantem Verhältnis entsprechend dem Wachstum der Pflanze der Lösung entnommen. Anders sehen die Kurven aus, bei denen P-arm angezogene Pflanzen auf P-reiche Versuchslösungen gesetzt wurden (Abb. 6, die beiden oberen Kurven). Hier ist der Isotopenaustausch durch zwei Effekte überlagert worden. Einmal ist der P-Gehalt dieser Pflanzen

viel geringer als in den vorher besprochenen Fällen und damit werden natürlich auch die austauschenden Mengen geringer. Zum anderen führt das hohe Konzentrations-Gefälle zwischen Versuchslösung und Pflanzen zu einer stärkeren Aufnahme als in den oben besprochenen Beispielen. Beide Effekte zusammen bewirken, daß der sicher auch bei diesen Versuchsgliedern stattfindende Austausch innerhalb des Meßfehlerbereiches liegt und so nicht nachweisbar ist.

Am eindruckvollsten läßt sich die Dynamik der Phosphoraufnahme durch die Pflanze jedoch an den Versuchen demonstrieren, bei denen P-reiche Pflanzen auf eine P-arme Versuchslösung gesetzt wurden (Abb. 7). In diesen Fällen kommt es aufgrund der Gleichgewichtseinstellung zwischen Pflanze und Lösung zunächst zu einer Abgabe von P aus der Pflanze an die Lösung – der Gehalt der Lösung an P^{31} steigt über die Ausgangskonzentration. Gleichzeitig nimmt der P^{32}-Gehalt der Lösung aber schon ab. Hier sind also gerade in der ersten Phase des Versuches die beiden Teilchenströme zwischen Pflanze und Lösung nebeneinander sichtbar. Sobald sich P^{31} und P^{32} gleichmäßig über beide Phasen verteilt haben, ist wieder nur noch die durch den Bedarf der Pflanze bedingte Nettoaufnahme nachweisbar.

Alle hier dargestellten Versuchsergebnisse sind in ihrer Größenordnung und Tendenz reproduzierbar. Abweichungen ergeben sich aus der individuellen Streubreite der Versuchspflanzen und den Veränderungen in den Klimafaktoren während Anzucht und Versuch.

DISKUSSION

Die Versuchsergebnisse zeigen, daß die Nährstoffaufnahme sehr viel dynamischer ist als bisher gemeinhin angenommen wurde. Nach den hier gewonnenen Ergebnissen ist wenigstens der erste Schritt der Nährstoffaufnahme ein Prozeß, bei dem sich ständig gleichartige – wenn auch nicht gleich große-Nährstoffströme in beide Richtungen bewegen. Die Größe dieser Bruttoaufnahme und -abgabe ist von den herrschenden Konzentrationen in den Phasen abhängig, die Differenz zwischen beiden ist die mit konventionellen Methoden meßbare Nettoaufnahme oder -abgabe. Diese Nettoaufnahme ist letztlich eine Folge des Wachstums, durch das das Ionengleichgewicht zwischen Pflanze und Nährlösung immer wieder gestört wird.

Sobald das Wachstum aufhört, stagniert auch die Nährstoffaufnahme, da nun - wie in jedem Mehrphasensystem im Gleichgewicht - die Nährstoffströme in beiden Richtungen gleich groß sind. Abbauvorgänge in der Pflanze führen dagegen ebenso wie alle anderen Verhältnisse, bei denen die Innenkonzentration höher wird als die Außenkonzentration, zu einer Phosphorabgabe an die Lösung.

Wenn diese Ausführungen auch zunächst nur speziell für das verwendete Versuchsmaterial unter den gewählten Bedingungen gelten, so darf doch angenommen werden, daß es sich hier um ein verbreitetes Prinzip handelt. Diese Ansicht wird durch neuere Versuche mit Mais gestützt.

Eine mathematische Auswertung der hier vorgelegten und weiterer Versuche, die einen tieferen Einblick in die Kinetik des Phosphataustausches und der Phosphataufnahme gestattet, soll in einer weiteren Veröffentlichung erfolgen.

ZUSAMMENFASSUNG

Ausgehend von der bekannten Tatsache, daß Pflanzen unter bestimmten Umständen Nährstoffe durch die Wurzeln abgeben können, wurde zunächst untersucht, inwieweit P-Aufnahme und P-Abgabe von dem Verhältnis der Nährstoffkonzentrationen in Pflanze und Nährlösung abhängt. Dabei zeigte sich, daß junge Haferpflanzen unter den gewählten Bedingungen an eine P-freie Nährlösung laufend Phosphor abgeben, während sie schon bei wenig mehr als 0,2 mg P/l ausreichend versorgt sind. Bei weiterer Erhöhung der P-Konzentration in der Lösung wird nicht mehr Trockensubstanz gebildet. Ab etwa 5 mg P/l steigt auch die P-Aufnahme nicht mehr weiter. Je P-reicher die Pflanzen angezogen sind – je höher damit ihr P-Gehalt ist – desto P-reicher ist auch ihre Gleichgewichtslösung, d.h. die Nährlösung, bei der weder P-Aufnahme noch P-Abgabe durch die Pflanze stattfindet. Die Beziehung zwischen Gleichgewichtslösung einerseits und Anzuchtlösung bzw. P-Gehalt der Pflanze auf der anderen Seite ist nicht linear. Der Einsatz von Isotopen zeigte weiter, daß mindestens der erste Schritt der Nährstoffaufnahme ein Diffusionsprozeß ist, bei dem sich Ströme der gleichen Ionenart in beide Richtungen bewegen. Die mit konventionellen Methoden meßbare Aufnahme bzw. Abgabe ist also der aus einer Bruttoaufnahme und Bruttoabgabe resultierende Nettobetrag, dessen Vorzeichen und Größe von der Größe der beiden Bruttobewegungen und damit von den Konzentrationen in der Pflanze und der Lösung bestimmt wird.

Eingegangen am 10. September 1960

LITERATUR

1 Briggs, G. E. and Robertson, R. N., Apparent free space. Ann. Rev. of Plant Physiol. **8**, 11–30 (1957).
2 Laatsch, W., Dynamik der mitteleuropäischen Mineralböden. Th. Steinkopf, Dresden-Leipzig (1954).
3 Laties, G. G., Active transport of salt into plant tissue. Ann. Rev. Plant Physiol. **10**, 87 ff (1959).
4 Lundegardh, H., Investigations on the mechanism of absorption and accumulation of salts V. Physiol. Plantarum **12**, 342–352 (1959).
5 Michael, G. und Marschner, H., Über den Phosphataustausch an Wurzeloberflächen. Z. Botanik **46**, 37–52 (1958).
6 Scott Russell, R., Tracer methods with isotopes. Handb. Pflanzenphysiol. IV., 100–107, Springer Verlag Berlin (1958).
7 Wrangell, M. von, Die Bestimmung der pflanzenzugänglichen Nährstoffe des Bodens. Landw. Jahrb. Z. f. wiss. Landw. **71**, 149 ff, (1930).

Plant and Soil XIV, no. 3 May 1961

LE PROBLÈME DE L'EXCRÉTION D'AZOTE PAR LES LÉGUMINEUSES

PREMIÈRES RECHERCHES EN RÉGION ÉQUATORIALE

par J. R. SEEGER

Division d'Agrologie de l'I.N.E.A.C., Yangambi, Congo

SOMMAIRE

I. Introduction

II. Resultats expérimentaux
A. Substrat avec faibles réserves azotées.
B. Substrat sans azote.
C. Influence de la lumière.
D. Influence de la durée de végétation.

III. Discussion des résultats
Résumé – Summary.
Bibliographie.

I. INTRODUCTION

La culture en association de légumineuses et de non-légumineuses est une pratique très ancienne, que l'on retrouve dans la plupart des systèmes primitifs d'agriculture. Plusieurs exemples de telles associations peuvent être trouvés en Malaisie, à Ceylon et aux Indes; c'est un système qui est également pratiqué par les Indiens d'Amérique du Nord (Nicol [7]).

En Afrique également les exemples sont nombreux. Au Ruanda-Urundi, les populations rurales cultivent haricots (*Phaseolus vulgaris*), maïs (*Zea maïs*), patates douces (*Ipomoea batatas*); sorgho (*Sorghum vulgare*), manioc (*Manihot esculenta*) et pois (*Pisum sp.*) en association de 3 ou 4 espèces suivant les régions et les circonstances. En Angola, il n'y a pas de rotation en agriculture indigène; les légumineuses sont toujours associées à d'autres espèces non-légumineuses, généralement le maïs (Coutinho [2]).

Cette pratique culturale s'est également répandue dans les systèmes intensifs d'agriculture, mais sous des formes plus rationnelles.

Si l'on reconnaît presque unanimement l'intérêt de cette méthode qui permet à la non-légumineuse de se développer mieux que si elle était cultivée seule, l'origine de cet effet bénéfique a fait l'objet de nombreuses recherches et controverses.

Lipman [4], en 1912, fut un des premiers à se pencher sur ce problème; ses travaux aboutirent à la conclusion que des Légumineuses bien nodulées pouvaient excréter de l'azote dont bénéficiaient les non-Légumineuses qui leur étaient associées. D'autres chercheurs n'arrivèrent pas à confirmer les hypothèses de Lipman et ses travaux restèrent dans l'oubli pendant une vingtaine d'années. C'est en 1933 que Virtanen et ses collaborateurs, par une série d'expériences très rigoureuses, purent démontrer, sans aucun doute possible, que certaines légumineuses sont capables d'excréter de l'azote dans le sol, en cours de croissance (Wilson et Burton [14]). Cet azote est excrété par des jeunes nodules sous forme d'acide aspartique; il peut être utilisé directement par une non-Légumineuse associée, sans aucune transformation préalable (Virtanen et coll. [12]).

Les travaux de Virtanen et collaborateurs eurent un grand retentissement et soulevèrent à travers le monde un véritable courant de recherches. Des résultats contradictoires étant apparus, ils donnèrent naissance à une vaste polémique.

Parmi les travaux les plus importants ayant abouti à la conclusion indiscutable que les légumineuses pouvaient excréter de l'azote, citons ceux de Virtanen et collaborateurs [12 13] en Finlande, ceux de Thornton et Nicol [11] en Angleterre et ceux de Madhok [6] aux Indes. D'autres auteurs arrivèrent à des résultats variables, positifs dans certains cas mais négatifs dans d'autres; parmi ceux-ci: Nowotnowna [8] en Écosse et Pologne, Wilson et Wyss [15] et Wilson et Burton [14] aux États-Unis et Scholz [9] en Allemagne. D'autres auteurs par contre n'ont jamais pu, avec certitude, mettre cette excrétion d'azote en évidence et parmi eux: Ludwig et Allison [5] aux États-Unis, Engel et Roberg [3] en Allemagne, Bond et Boyes [1] en Écosse et Swaby [10] en Australie. De l'ensemble de ces travaux, il apparaît que l'excrétion d'azote est un phénomène possible mais non habituel qui nécessite pour se manifester la réalisation de conditions particulières, mal définies jusqu'à présent.

De tous les facteurs qui interviennent dans l'excrétion, il semble que ce soit le rapport C/N dans la plante le plus important. Il en découle que les conditions écologiques seront déterminantes et parmi celles-ci le rôle principal a été attribué aux facteurs lumière et température. Il est toutefois impossible d'en fixer les conditions optimales car si Bond et Boyes [1] attribuent leurs résultats négatifs à une lumière insuffisante, Wilson et Wyss [15] cependant arrivent à des résultats positifs en ombrageant leurs cultures. Virtanen [12] avait également attribué une certaine importance à l'humidité

atmosphérique, mais aucune influence de ce facteur n'a pu être mise en évidence dans les essais de Swaby [10]. Virtanen et ses collaborateurs [12] ont démontré que les propriétés adsorbantes du substrat influençaient fortement le taux d'excrétion. Dans le cas des cultures associées c'est la non-légumineuse qui fait office d'adsorbant et ce pouvoir varie fortement d'une espèce à l'autre.

Il ne semble pas que toutes les légumineuses jouissent des mêmes facultés d'excréter de l'azote. La grande majorité des résultats positifs a été obtenue avec des légumineuses cultivées en régions tempérées (pois, trèfles, vesces...) tandis que la plupart des essais réalisés avec des légumineuses tropicales se sont soldés par un échec. Les raisons de cette différence ne sont pas connues (Wilson et Wyss [15]). L'intensité de l'excrétion dépendrait également de la souche de *Rhizobium* ayant colonisé les racines. Les nodules formés par certaines souches hautement "effectives" excrètent peu d'azote tandis que d'autres, issus de souches aux qualités fixatrices moindres, peuvent excréter beaucoup plus d'azote; il n'y a donc aucune corrélation entre l'"effectivité" d'une souche et l'excrétion d'azote (Virtanen et coll. [12]).

Bien d'autres facteurs jouent encore sur ce phénomène: les dimensions du vase de végétation, le nombre de plants par vase de végétation, le pourcentage de légumineuses par rapport aux non-légumineuses, la nature du substrat, la durée de l'expérience, ... *etc.*

Tant de conditions doivent être remplies pour qu'il y ait excrétion que Wilson et Burton [14] estiment qu'il serait peu sage de la considérer comme un phénomène habituel et qu'il vaudrait mieux la considérer comme un fait exceptionnel.

Il serait encore plus hasardeux d'après Wilson et Burton de vouloir conclure à la suite de ces résultats controversés et obtenus en conditions artificielles, que l'excrétion doit avoir lieu dans les conditions de la pratique courante. Ces auteurs se demandent s'il ne s'agit pas plutôt d'un "greenhouse artifact".

Il est possible toutefois qu'elle puisse intervenir en champ dans certaines conditions, mais il n'est techniquement pas possible de la mettre en évidence avec certitude.

Si la question de l'excrétion est fortement controversée, la plupart des auteurs reconnaissent cependant que les légumineuses peuvent apporter de l'azote aux non-légumineuses qui leur sont associées par la décomposition des nodules et racines.

Bien d'autres facteurs peuvent encore être invoqués pour expliquer les raisons de l'association bénéfique de légumineuses avec des non-légumineuses, mais ceci n'entre pas dans le but de cette note.

A notre connaissance, aucun essai de cultures associées de légumineuses et de non-légumineuses n'avait été entrepris jusqu'à présent en milieu équatorial dans le but d'étudier l'excrétion d'azote. C'est pourquoi, nous avons entrepris des essais à Yangambi (24°29′ E.; 0,49° N.) en région forestière équatoriale, afin de déterminer si dans

ces conditions écologiques, très différentes de celles étudiées jusqu'à présent, certaines légumineuses pouvaient excréter de l'azote.

II. RESULTATS EXPERIMENTAUX

Réalisation technique

Tous les essais furent réalisés en abri de Mitscherlich; les vases de végétation, placés sur des tables roulantes, étaient exposés en lumière naturelle durant la journée et mis sous abri vitré la nuit ou en cas de pluie.

Différents types de vase de végétation furent utilisés: des pots en fer de 2 litres, des vases de végétation en fer ou émaillés de 6 litres et des seaux de 10 litres.

Deux types de substrat furent employés. Dans une première série d'essais, on utilisa un mélange de 1/5 de terre de culture très légère (4–5% d'argile) et 4/5 de sable assez fin, provenant des alluvions d'un tributaire du fleuve Congo, dépourvu de minéraux altérables. Dans la seconde série, le sable fut utilisé à l'état pur.

Les semences furent désinfectées au sublimé corrosif avant le semis.

Le nombre total de plants par vase de végétation était fonction des dimensions de celui-ci et des espèces cultivées. La proportion de légumineuses et de non-Légumineuses fut toujours de 1/1.

Les arrosages furent effectués avec de l'eau de pluie, bouillie pendant 20 minutes, à l'aide de récipients stérilisés. Une fois par semaine, les plants étaient arrosés avec la solution minérale sans azote, utilisée par Nowotnowna[8] à Rothamsted.

La durée des essais fut en moyenne de 6 à 8 semaines. Deux considérations déterminèrent cette durée; d'après Virtanen et ses collaborateurs [12 13] l'excrétion d'azote serait optimum pendant le jeune âge des nodules, avant la floraison.

D'autre part, la plupart des légumineuses étudiées ont un cycle de végétation très court, de 3 mois environ. Nous avons craint, en allongeant la durée de l'essai, de confondre excrétion et libération d'azote par des nodosités en voie de décomposition, étant donné la brièveté du cycle végétatif et le renouvellement partiel de la nodulation en cours de cycle. Ce point fut d'ailleurs vérifié.

Cinq objets furent réalisés dans chaque essai:

– Légumineuse non inoculée
– Légumineuse inoculée
– Non-légumineuse
– Non-légumineuse + légumineuse non inoculée
– Non-légumineuse + légumineuse inoculée.

C'est la non-légumineuse des deux derniers objets qui servit de test, permettant de déceler s'il y avait oui ou non excrétion d'azote par la légumineuse associée. Afin de ne pas surcharger les tableaux, nous en avons écarté les

résultats d'analyse concernant la légumineuse et ceux de la non-légumineuse en culture pure. Les données concernant la légumineuse ont un intérêt moindre dans le problème envisagé. Les chiffres concernant la non-légumineuse en culture pure étaient du même ordre que ceux concernant la non-légumineuse associée à une légumineuse non inoculée et n'apportaient aucun élément nouveau. De plus, il nous a semblé plus intéressant de mettre en comparaison des résultats de non-légumineuses placées en conditions identiques sauf en ce qui concerne la présence ou l'absence de nodulation. Le nombre de répétitions variait d'un essai à l'autre d'après la dimension du vase de végétation; il y en avait 5 pour les seaux de 10 litres et 11 pour les pots de 2 litres, dans lesquels le nombre de plants était moindre.

L'inoculation fut réalisée à l'aide d'une suspension dense d'une souche spécifique et "effective" de Rhizobium. Au total 18 expériences furent réalisées.

A. *Substrat avec faibles réserves azotées* (Tableau 1)

Une faible dose d'azote fut introduite dans les substrats, soit en mélangeant 1/5 de terre de culture avec 4/5 de sable, soit en ajoutant un peu d'azote lors du premier arrosage du sable pur. Le but de ce faible apport azoté était de permettre un développement satisfaisant de la non-légumineuse.

Six essais furent réalisés. Dans deux d'entre eux une nodulation assez abondante et "effective" s'était installée dans les objets non inoculés; leurs résultats ne seront pas donnés pas ici.

Seul l'essai Mucuna (*Stizolobium deeringianum*) — mais marqué d'une astérisque, fut réalisé sur sable pur avec addition de 50 mg d'azote au départ; dans les autres essais le substrat était composé d'un mélange de 4/5 de sable avec 1/5 de terre de culture.

Dans 3 des 4 essais, la non-légumineuse a tiré un bénéfice très net de son association avec une légumineuse bien nodulée. Une ex-

TABLEAU 1

Association	Durée de l'essai (jours)	Poids sec (P.S.) et teneur en azote des parties aériennes de la non-légumineuse								
		Non inoculé			Inoculé			Inoculé en % du non inoculé		
		P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot
Arachide – maïs	59	13,9	0,50	69,5	20,3	0,46	93,38	146,4	92	134,3
Mucuna – maïs *	52	12,8	0,55	70,4	20,4	0,49	99,96	159,3	89	141,9
Mucuna – maïs	64	2,5	0,51	12,75	2,8	0,65	18,20	112,0	130	142,7
Mucuna – *Urena lobata* . . .	40	2,2	0,81	17,82	1,7	0,91	15,47	77,2	112	86,8

plication très plausible apparait aussitôt: en l'absence de nodulation, la légumineuse et la non-légumineuse doivent se partager le peu d'azote disponible dans le substrat; au contraire, lorsque la légumineuse est bien nodulée, elle utilise l'azote de l'air et permet à la non-légumineuse de disposer à elle seule de la presque totalité de l'azote du substrat. Dans un commentaire des travaux de Lipman, Wilson et Burton [14] arrivent à des conclusions analogues.

Même s'il y a excrétion, l'utilisation d'un substrat contenant de l'azote ne permet de tirer aucune conclusion certaine; de plus, deux des essais ont échoué à cause de salissages apportés par la terre, c'est pourquoi la technique du substrat mélangé fut abandonnée dans les essais ultérieurs.

B. *Substrat sans azote* (Tableau 2)

Le substrat est constitué uniquement du sable décrit précédemment. Les arrosages sont effectués avec une solution minérale sans azote. Toutes les expériences ont été réalisées en pots de fer de 2 litres. Après remplissage ils étaient soumis à 2 stérilisations d'une heure à 121°, à 24 heures d'intervalle.

Le maïs a tiré un gain d'azote de son association avec arachide (*Arachis hypogaea*) et soja (*Glycine max.*). Un examen minutieux des racines de ces deux légumineuses n'a pas permis de déceler des nodules en voie de dégradation; on peut donc raisonnablement songer, dans ces deux essais, à une excrétion d'azote par les nodosités du soja et de l'arachide.

Dans les associations avec *Vigna sinensis* et *Voandzeia subterranea*, le maïs, malgré des teneurs en N plus élevées, a souffert au total de

TABLEAU 2

Poids sec et teneur en azote des parties aériennes de la non-légumineuse										
Association	Durée de l'essai (jours)	Non inoculé			Inoculé			Inoculé en % du non inoculé		
		P.S. en g, m̄/pot	N en %, m̄/pot	N en mg m̄/pot	P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g, m̄/pot	N en %, m̄/pot	N en m m̄/pc
Arachide — maïs .	43	0,38	0,75	2,85	0,39	0,84	3,28	102,6	112,0	115,
Soja — maïs . . .	43	1,17	0,74	8,66	1,40	0,72	10,08	119,6	97,2	116,
Vigna — maïs . .	45	1,14	0,70	7,98	0,87	0,75	6,52	75,4	105,7	79,
Voandzeia — maïs	53	1,28	0,66	8,45	1,13	0,71	8,02	88,0	107,5	94,

son association avec des légumineuses cependant très bien nodulées. Dans le cas du Voandzeia, cet effet dépressif est probablement dû au fait que les plants inoculés étaient beaucoup plus vigoureux que les non inoculés et la concurrence avec le maïs était par conséquent beaucoup plus forte dans le premier cas. Pour le Vigna par contre, il y avait peu de différences entre les objets inoculés et non inoculés, l'explication précédente ne peut donc entrer en ligne de compte et le fait demeure peu compréhensible.

Deux essais avec *Crotalaria longithyrsa* et un essai avec *Phaseolus mungo* ont été abandonnés suite à une mauvaise croissance de la légumineuse.

Addendum: Plusieurs expériences ont été tentées avec des légumineuses de régions tempérées: *Pisum sativum*, *Lupinus sp.* et *Phaseolus vulgaris;* leur croissance étant généralement mauvaise, nous n'avons pu en tenir compte.

C. *Influence de la lumière* (Tableau 3)

Ainsi qu'il a été vu précédemment, la lumière serait un facteur important qui conditionne les possibilités d'excrétion d'azote par une légumineuse. Si certains auteurs ont attribué leurs échecs à une lumière insuffisante, d'autres par contre, ont pu obtenir des résultats positifs en ombrageant leurs essais (Wilson et Wyss [15]).

Un essai fut entrepris dans ce sens à Yangambi avec l'association arachide - maïs. L'expérience fut réalisée en pots de 2 litres sur sable pur.

Deux séries comportant les objets habituels furent établies. Pour la première série, on appliqua le mode courant: les pots étaient

TABLEAU 3

Association arachide—maïs (43 jours de végétation)		Poids sec et teneur en azote des parties aériennes des 2 membres de l'association								
		Non inoculé			Inoculé			Inoculé en % du non inoculé		
		P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g	N en %	N en mg
Maïs	Lumière du jour + abri . . .	0,38	0,75	2,85	0,39	0,84	3,28	102,6	112,0	115,1
	Abri permanent	0,46	0,70	3,01	0,38	0,78	2,94	88,3	111,4	97,7
Ara-chide	Lumière du jour + abri . . .	1,36	1,38	18,6	2,23	3,91	86,9	163,9	283,3	467,2
	Abri permanent	1,54	1,57	24,1	2,29	3,35	77,1	148,7	213,3	319,9

placés en lumière naturelle pendant la journée et mis sous abri la nuit ou en cas de pluie. Les pots de la seconde série par contre restèrent en permanence sous abri.

Lorsque la légumineuse n'est pas nodulée, une réduction de la luminosité a un effet favorable sur les poids sec et l'immobilisation d'azote de l'arachide et du maïs. Au contraire si l'arachide est bien nodulée, c'est dans la série sous abri permanent que l'on trouve les teneurs et immobilisations d'azote les plus basses. A en juger par l'immobilisation azotée dans les parties aériennes du maïs, l'excrétion d'azote ne se manifeste que lorsque les plants sont placés en lumière naturelle, elle est nulle sous abri permanent.

La réduction de luminosité joue donc ici dans un sens défavorable; il aurait été intéressant d'étudier, toutes autres conditions restant égales, quel était l'effet d'un complément de lumière. Nous ne disposons malheureusement pas du matériel nécessaire pour cette expérience.

D. *Influence de la durée de végétation* (Tableaux 4 et 5)

Deux expériences furent entreprises, la première avec l'association soja—maïs, la seconde avec l'association arachide—maïs.

Le premier essai fut réalisé en seaux de 10 litres sur sable pur; il comportait les objets habituels. Une première moitié de l'expérience fut arrêtée à 42 jours, la seconde moitié à 76 jours.

L'expérience arachide—maïs réunit deux essais espacés dans le temps qui furent réalisés en pots de 2 litres sur sable pur, stérilisés après remplissage.

D'après Virtanen et collaborateurs [12] l'excrétion d'azote serait maximum chez des nodules jeunes avant la floraison de la plante.

TABLEAU 4

Poids sec et teneur en azote des parties aériennes du maïs									
Association soja—maïs	Non inoculé			Inoculé			Inoculé en % du non inoculé		
	P.S. en g, m̄ de 10 plants	N en %	N en mg, m̄ de 10 plants	P.S. en g, m̄ de 10 plants	N en %	N en mg, m̄ de 10 plants	P.S. en g	N en %	N en mg
Durée de végétation de 42 jours . . .	2,04	0,89	18,16	2,17	0.57	12,39	106,3	64,1	68,2
Durée de végétation de 76 jours . . .	4,71	0,56	26,38	4,73	0,69	32,43	100,4	123,2	123,7

TABLEAU 5

Association arachide—maïs	Poids sec et teneur en azote des parties aériennes du maïs								
	Non inoculé			Inoculé			Inoculé en % du non-inoculé		
	P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g, m̄/pot	N en %, m̄/pot	N en mg, m̄/pot	P.S. en g	N en %	N en mg
Durée de végétation de 35 jours . . .	1,22	1,01	12,32	1,07	1,06	11,34	87,7	105,0	92,0
Durée de végétation de 43 jours . . .	0,38	0,75	2,85	0,39	0,84	3,28	102,6	112,0	115,1

Le premier essai fut arrêté à 35 jours, durée qu'il était difficile de raccourcir; rappelons en effet que la nodulation de l'arachide s'installe 12 à 14 jours après le semis et que la floraison apparaît 28 jours après le semis. Le second essai fut arrêté après une durée de végétation de 43 jours.

Les fortes différences de poids sec entre les deux essais sont dues au fait que le nombre de plants par pot était double dans le premier essai.

Ces deux expériences mettent clairement en évidence l'influence considérable de la durée de végétation sur les résultats obtenus.

Dans la première expérience, le maïs a tiré un bénéfice assez net de son association avec le soja inoculé, après 76 jours de végétation. Il serait cependant très hasardeux d'en conclure qu'il s'agit là d'un phénomène d'excrétion; il est plus que vraisemblable que ce gain d'azote provient de la décomposition des nodules du soja, proche de sa maturité.

Dans la deuxième expérience, on constate une excrétion probable d'azote pour une durée de végétation de 43 jours, elle est nulle ou non décelable après 35 jours. Il y a donc un minimum de durée de végétation en-dessous duquel on ne peut descendre, ce minimum se situe aux environs de 6 semaines.

Les observations de Virtanen et collaborateurs [12] avaient été faites sur des espèces de régions tempérées à cycle de végétation long et floraison tardive; l'arachide par contre, boucle son cycle en 3 mois et fleurit à 28 jours; l'obtention de résultats divergents n'a donc rien d'étonnant.

III. DISCUSSION DES RESULTATS

1. De l'ensemble des expériences réalisées, deux seulement tendent à prouver que certaines légumineuses peuvent excréter de l'azote dans les conditions écologiques de Yangambi. Les gains obtenus sont nets mais n'ont pas le caractère spectaculaire des résultats obtenus par Virtanen et collaborateurs [12 13]. Nous n'oserions affirmer que ces résultats obtenus en vases de végétation, peuvent être transposés aux conditions normales de culture.

2. Si l'excrétion d'azote n'est pas apparue, dans nos essais, comme un phénomène certain et important, l'intérêt de l'association de légumineuses et de non-légumineuses s'est manifesté cependant sous d'autres aspects. Grâce à son association avec une légumineuse bien nodulée, la non-légumineuse peut disposer, à elle seule, de la presque totalité de l'azote assimilable du sol. De plus, en fin de végétation la dégradation des nodules libère une quantité importante d'azote dont profite la non-légumineuse. On peut raisonnablement supposer qu'il en est de même dans les conditions de la pratique courante; comme la dégradation des nodules est plus précoce et plus rapide en conditions naturelles, il est probable que cet effet bénéfique soit même plus élevé en champ.

3. L'étude des conditions de luminosité a montré qu'une réduction de celles-ci avait un effet défavorable sur l'excrétion et qu'il serait, au contraire, probablement plus intéressant en conditions expérimentales d'augmenter l'intensité lumineuse ou de prolonger la longueur de jour.

4. La durée optimum des expériences semble se situer entre 6 et 7 semaines de végétation. En deça de cette période, aucune excrétion n'a pu être décelée; si l'on prolonge par contre la durée de végétation, un bénéfice net apparaît, provenant en majeure partie de la libération d'azote par des nodules en voie de décomposition. Chez l'arachide par exemple, cette dégradation s'amorce vers la septième semaine, les vieux nodules volumineux se désagrègent et sont remplacés au fur et à mesure par de jeunes nodules.

RESUMÉ

Des essais de cultures associées de légumineuses et de non-légumineuses ont été entrepris à Yangambi (Congo), en vases de végétation, dans le but d'étudier si les légumineuses pouvaient excréter de l'azote en régions équatoriales.

Deux essais seulement sur 18 permirent de déceler une excrétion probable d'azote.

Dans les autres essais, ou bien la non-légumineuse ne tirait aucun bénéfice de l'association ou bien elle accusait un gain considérable d'azote mais ne provenant pas de l'excrétion.

Une réduction de la luminosité naturelle n'a pas eu d'effet favorable sur l'excrétion dans les conditions de Yangambi.

La durée de végétation optimum des essais se situe entre 6 et 7 semaines.

SUMMARY

Associated cultures of legumes and non-legumes were grown at Yangambi (Congo), in pots, to test whether legumes do excrete nitrogen in the equatorial regions or not.

In only two of the eighteen trials it was possible to detect a probable nitrogen excretion.

In the other trials, either the non-legume did not take any benefit from its association or it took an appreciable nitrogen profit, which didn't originate from excretion.

The reduction of the natural light intensity had no favourable effect on excretion under the conditions of Yangambi.

The optimum growth period for those trials lies between 6 and 7 weeks.

Les analyses d'azote ont été effectuées par MM. R. De Meester, G. Quidousse, et A. Ronday au Laboratoire Central d'Analyses, sous la direction de Mr. J. Croegaert. Nous les en remercions très vivement.

Reçu le 29 juillet 1960

BIBLIOGRAPHIE

1 Bond, G. and Boyes, J., Excretion of nitrogenous substances from root nodules: Observations on various leguminous plants. Ann. Botany, N.S. **3**, 901–904 (1939).
2 Coutinho, Ir. L. P., Rapport de réunion F.A.O. 1958/22 (1958).
3 Engel, H. und Roberg, M., Die Stickstoffausscheidung der Wurzelknöllchen. Ber. Deut. Botan. Ges. **56**, 337–352 (1938).
4 Lipman, J. G., New Jersey Agr. Expt. Sta. Bull. **253** (1912) (cité par Wilson and Burton [14]).
5 Ludwig, C. A. and Allison, F. E., Experiments concerning diffusion of nitrogenous compounds from healthy legume nodules or roots. Botan. Gaz. **98**, 680–695 (1937).
6 Madhok, M. R., Association of legumes and non-legumes. Soil Sci. **49**, 419-432 (1940).
7 Nicol, H., Mixed cropping in primitive agriculture. Empire J. Exptl. Agr. **3**, 189–195 (1935).
8 Nowotnowna, A., An investigation of nitrogen uptake in mixed crops not receiving nitrogenous manure. J. Agr. Sci. **27**, 503–510 (1937).

9 Scholz, W., Zur Stickstoffernährung von-Gramineen im Mischanbau mit Leguminosen. Bodenk. u. Pflanzenernähr. **15**, 47–73 (1939).
10 Swaby, R. J., Do legumes excrete nitrogenous compounds? J. Australian Inst. Agr. Sci. **11**, 23–28 (1945).
11 Thornton, H. G. and Nicol, H., Further evidence upon the nitrogen uptake of grass grown with lucerne. J. Agr. Sci. **24**, 540–543 (1934).
12 Virtanen, A. I., Von Hausen, S., and Laine, T., Investigations on the root nodule bacteria of leguminous plants. XIX. Influence of various factors on the excretion of nitrogenous compounds from the nodules. J. Agr. Sci. **27**, 332–348 (1937).
13 Virtanen, A. L., Von Hausen, S. and Laine, T., Investigations on the root nodule bacteria of leguminous plants. XX. Excretion of nitrogen in associated cultures of Legumes and non-Legumes. J. Agr. Sci. **27**, 584–610 (1937).
14 Wilson, P. W. and Burton, J. C., Excretion of nitrogen by leguminous plants. J. Agr. Sci. **28**, 307–323 (1938).
15 Wilson, P. W. and Wyss, O., Mixed cropping and the excretion of nitrogen by leguminous plants. Soil Sci. Soc. Am. Proc. **2**, 289–297 (1937).

Plant and Soil XIV, no. 3 May 1961

ABHÄNGIGKEIT DER LÖSLICHKEIT DER PHOSPHORSÄURE UND DES KALIS IM BODEN VON DER POTENTIELLEN WASSERSTOFFIONENKONZENTRATION

von WERNER RATHJE *

Im Folgenden soll an Hand von Versuchen eine Erscheinung beschrieben werden, die für die Löslichkeit der Phosphorsäure und des Kalis im Boden von Bedeutung sein dürfte.

VERSUCHE

1. *Bestimmung der Löslichkeitsgleichgewichte des Hydroxylfluorapatits und der Austauschgleichgewichte von Aluminium- und Eisen-Hydroxydphosphat in Natriumacetat-Essigsäure-Pufferlösungen von verschiedener potentieller Wasserstoffionenkonzentration*

Hydroxylfluorapatit, das isomorphe Gemisch von 30% Hydroxylapatit und 70% Fluorapatit wurde nach der Methode der acidimetrischen Ausfällung in weitgehend reiner Form dargestellt [3] und nach mehrmaligem Abdekantieren und Auswaschen mit bidestilliertem, siedendem Wasser in Form einer Suspension von 200 mg (= 0,2 mMol) in 50 ccm Wasser erhalten. Weiterhin wurden die Austauschersubstanzen Aluminium- und Eisen-Hydroxydphosphat durch Zusammengeben von Aluminium- oder Eisen- und Phosphationen im molaren Verhältnis 15 : 1 bei neutral gehaltener Reaktion aus siedender Lösung ausgefällt [4] und nach mehrmaligem Abdekantieren und Auswaschen mit bidestilliertem, siedendem Wasser ebenfalls in Form von Suspensionen erhalten, die 9 mMol Al_2O_3 oder Fe_2O_3 und 0,6 mMol P_2O_5 je 50 ccm Wasser enthielten. Je 2 ccm dieser Suspensionen wurden zu 20 ccm bidestilliertem, kohlensäurefreiem Wasser zugesetzt, dessen Reaktion vorher durch Zufügen geringer Mengen $N/100$ Natronlauge mit Hilfe einer Glaselektrode auf den pH-Wert = 7 eingestellt worden war. Weiterhin wurden je 5 ccm der Suspensionen zu Proben von 20 ccm $N/8$, $N/80$ und $N/800$ Natriumacetatlösung zugesetzt, deren Reaktion vorher

* Institut für Zuckerindustrie der Technischen Universität Berlin, Berlin N65, Amrumstr. 32.

durch Zugabe von Salzsäure ebenfalls mit Hilfe einer Glaselektrode auf pH = 7 eingestellt worden war. Die Proben mit den Suspensionen befanden sich in Kölbchen mit eingeschliffenen Glasstopfen und wurden öfter umgeschüttelt. Wie in Parallelversuchen durch Bestimmung der Phosphatkonzentration nach verschiedenen Zeiten gefunden worden war, hatten sich in allen Fällen bereits nach 24 Stunden Gleichgewichte eingestellt. Nach 3 Tagen wurde in den Proben der pH-Wert und in aliquoten Teilen die Phosphatkonzentration (nach Filtrieren durch trockne Filter, Eindampfen und Abrauchen des Filtrates mit analysenreiner Schwefelsäure in Platinschalen) kolorimetrisch [5] bestimmt (Tabellen 1 und 2).

2. *Bestimmung der Löslichkeitsgleichgewichte des Hydroxylfluorapatits und der Austauschgleichgewichte von Aluminium- und Eisen-Hydroxydphosphat in Glykokoll-Lösungen*

Je 5 ccm der oben beschriebenen Suspensionen wurden zu Proben von 20 ccm *N*/8, *N*/80 und *N*/800 Glykokoll-Lösung zugesetzt, die einen pH-Wert = 7,4 aufwiesen. Nach öfterem Umschütteln wurde nach 3 Tagen der pH-Wert und in aliquoten Teilen die Phosphatkonzentration, wie oben angegeben, gemessen (Tabelle 2).

3. *Bestimmung der Austauschgleichgewichte eines Tones in Wasser und Glykokoll-Lösungen*

Hunderd Gram eines lufttrocknen Tones (T-Wert: 72 mval/100 g, S-Wert: 53 mval/100 g) wurden mit Wasser mehrmals auf der Zentrifuge gewaschen und in einem Liter Wasser suspendiert, dessen pH-Wert vorher durch Zufügen von wenig Natronlauge auf 7,4 eingestellt worden war. Je 50 ccm dieser Suspension wurde zu 50 ccm *N*/5, *N*/50 und *N*/500 Glykokoll-Lösungen zugesetzt, die einen pH-Wert von 7,4 besaßen. Nach öfterem Umschütteln wurden nach 10 Tagen der pH-Wert und in aliquoten Teilen die Kaliumkonzentration nach Abzentrifugieren flammenphotometrisch bestimmt (Tabelle 3).

ERGEBNISSE

Aus Tabelle 1 geht hervor:

a. Im Löslichkeitsgleichgewicht des Hydroxylfluorapatits nehmen die Konzentrationen an gelöstem Phosphat mit steigender potentieller Wasserstoffionenkonzentration zu.

b. Im Austauschgleichgewicht der Aluminium- und Eisen-Hydroxydphosphate nehmen die Konzentrationen an gelöstem Phosphat mit steigender potentieller Wasserstoffionenkonzentration zu.

c. Die Reaktion wurde in allen Proben durch die Einwirkung von Hydroxylfluorapatit nach der alkalischen Seite, durch die Einwir-

TABELLE 1

Phosphatkonzentrationen und pH-Werte im Gleichgewicht von Hydroxylfluorapatit sowie Aluminium- und Eisen-Hydroxydphosphat mit Wasser und mit *N*/1000, *N*/100, *N*/10 Natriumacetat-Essigsäure-Pufferlösungen (pH-Wert vorher: 7,0)

Phosphatmineralien		Wasser	Natriumacetat-Essigsäure-Pufferlösung		
			N/1000	*N*/100	*N*/10
a. Hydroxylfluorapatit	mg P_2O_5/l	0,2	1,0	6	19
	pH nachher	7,6	7,5	7.4	7,4
b. Aluminium-hydroxydphosphat	mg P_2O_5/l	0,3	0,8	3,0	9
	pH nachher	6,9	6,7	6,6	6,7
c. Eisen-hydroxydphosphat	mg P_2O_5/l	0,1	0,5	1,8	5
	pH nachher	7,0	6,8	6,8	6,8

kung von Aluminium- und Eisen-Hydroxydphosphat nach der sauren Seite hin verschoben.

Aus Tabelle 2 ist ersichtlich:

a. Das Löslichkeitsgleichgewicht des Hydroxylfluorapatits und die Austauschgleichgewichte der Aluminium- und Eisen-Hydroxydphosphate nehmen mit steigender Glykokoll-Konzentration zu.

b. Durch Einwirkung des Hydroxylfluorapatits auf die Glykokoll-Lösungen wird die Reaktion nach der alkalischen Seite, durch Einwirkung der Aluminium- und Eisen-Hydroxydphosphate nach der sauren Seite hin verschoben.

TABELLE 2

Phosphatkonzentrationen und pH-Werte im Gleichgewicht von Hydroxylfluorapatit sowie Aluminium- und Eisen-Hydroxydphosphat mit Wasser und mit *N*/1000, *N*/100, *N*/10 Glykokoll-Lösungen (pH-Wert vorher: 7,4)

Phosphatmineralien		Wasser	Glykokoll-Lösung		
			N/1000	*N*/100	*N*/10
a. Hydroxylfluorapatit	mg P_2O_5/l	0,2	0,8	4	14
	pH nachher	7,5	7,6	7,6	7,5
b. Aluminium-hydroxydphosphat	mg P_2O_5/l	0,3	0,9	3,3	10
	pH nachher	7,1	7,2	7,2	7,2
c. Eisen-hydroxydphosphat	mg P_2O_5/l	0,1	0,5	2,0	6
	pH nachher	7,2	7,1	7,2	7,2

TABELLE 3

Kaliumkonzentrationen und pH-Werte im Gleichgewicht eines Tones mit Wasser und mit $N/1000$, $N/100$, $N/10$ Glykokoll-Lösungen (pH-Wert vorher: 7,4)

Kalimineral	Wasser	Glykokoll-Lösung		
		$N/1000$	$N/100$	$N/10$
Ton mg K_2O/l	5	10	30	60
pH nachher	7,7	7,6	7,6	7,5

Tabelle 3 zeigt:

a. Die in den Austauschgleichgewichten des Tones befindlichen Kaliumkonzentrationen sind um so höher, je größer die Glykokoll-Konzentrationen sind.

b. Durch Einwirkung des Tones auf Wasser und auf die Glykokoll-Lösungen wird die Reaktion nach der alkalischen Seite hin verschoben.

DISKUSSION DER VERSUCHSERGEBNISSE

1. *Ursachen der Erhöhung der Phosphat- und Kaliumkonzentrationen im Löslichkeits- bzw. Austauschgleichgewicht von Phosphat- und Kaliumnineralien mit steigender potentieller Wasserstoffionenkonzentration*

a. Umsetzung von tertiärem Calciumphosphat* mit Wasser, Natriumacetat–Essigsäure-Pufferlösung und Glykokoll-Lösung.

Die Erhöhung der Löslichkeit von Calciumphosphat mit steigender potentieller Wasserstoffionenkonzentration (Tabelle 1, a) dürfte auf folgenden Umsetzungen beruhen: Bei Berührung von tertiärem Calciumphosphat mit reinem Wasser (pH = 7) gehen primär Ca^{++}- und PO_4^{---}-Ionen aus dem festen Zustand in den gelösten Zustand über. Hierbei werden die PO_4^{---}-Ionen durch Umsetzung mit Wasser gleichgewichtsmäßig in HPO_4^{--}- und $H_2PO_4^{-}$-Ionen sowie H_3PO_4 umgewandelt:

$$\begin{aligned} PO_4^{---} + HOH &\rightleftharpoons HPO_4^{--} + OH^- \\ HPO_4^{--} + HOH &\rightleftharpoons H_2PO_4^{-} + OH^- \qquad (1) \\ H_2PO_4^{-} + HOH &\rightleftharpoons H_3PO_4 + OH^-. \end{aligned}$$

* Unter tertiärem Phosphat sei eine Verbindung verstanden, die PO_4^{---}-Ionen enthält, z.B. Hydroxylfluorapatit.

Die hierbei entstehenden Hydroxylionen bewirken, daß die Lösung des tertiären Calciumphosphates in Wasser alkalisch reagiert (wie allgemein die Lösung eines Salzes einer starken Base und einer schwächeren Säure). In reinem Wasser wird schon durch eine geringe Menge Hydroxylionen die (aktuelle) Hydroxylionenkonzentration so weit erhöht, daß Gleichgewichte nach Gleichung (1) erreicht werden, und eine PO_4^{---}-Ionenkonzentration entstanden ist, die dem Löslichkeitsprodukt entspricht. Die in diesen Gleichgewichten in Lösung befindlichen Calcium- und Phosphatkonzentrationen sind daher gering. – Bei Berührung von tertiärem Calciumphosphat mit Natriumacetat–Essigsäure-Pufferlösung von pH = 7 gehen ebenfalls primär Ca^{++}- und PO_4^{---}-Ionen in Lösung. Letztere werden wieder nach Gleichung (1) zum größten Teil in HPO_4^{--}- und $H_2PO_4^{-}$-Ionen sowie H_3PO_4 umgewandelt. Die gleichzeitig entstehenden Hydroxylionen werden jedoch zum größten Teil mit den von der Pufferlösung gelieferten Wasserstoffionen zu Wasser verbunden, sodaß die Umsetzung nach Gleichung (1) weiter von links nach rechts verlaufen kann. Erst wenn viel tertiäres Calciumphosphat in Lösung gegangen ist, ist die (aktuelle) Hydroxylionenkonzentration so weit erhöht (Tabelle 1, a), daß Gleichgewichte erreicht sind, und eine PO_4^{---}-Ionenkonzentration entstanden ist, die dem Löslichkeitsprodukt entspricht. – In gleicher Weise werden bei der Einwirkung von Glykokoll-Lösung auf tertiäres Calciumphosphat die nach Gleichung (1) entstehenden Hydroxylionen zum größten Teil mit Wasserstoffionen zu Wasser umgesetzt, sodaß die Löslichkeit von tertiärem Calciumphosphat in Glykokoll-Lösung gegenüber der Löslichkeit in Wasser ebenfalls stark erhöht ist (Tabelle 2, a).

b. Umsetzung der Anionenaustauschersubstanzen Aluminium- und Eisen-Hydroxydphosphat mit Wasser, Natriumacetat–Essigsäure-Pufferlösung und Glykokoll-Lösung.

Die Erscheinung der Erhöhung der Phosphatkonzentration in den Austauschgleichgewichten der Aluminium- und Eisen-Hydroxydphosphate mit steigender potentieller Wasserstoffionenkonzentration (Tabelle 1, b, c) dürfte auf ähnlichen Umsetzungen beruhen: Bei Berührung von Aluminium- und Eisen-Hydroxydphosphat mit reinem Wasser (pH = 7) werden OH^--Ionen (aus dem Wasser)

gegen PO_4^{---}-Ionen (aus dem Austauscher) ausgetauscht:

$$\begin{matrix} OH \\ Al\,OH \\ OH \\ Al\,PO_4 \end{matrix} + \begin{matrix} HOH \\ HOH \\ HOH \end{matrix} \rightleftharpoons \begin{matrix} OH \\ Al\,OH \\ OH \\ OH \\ Al\,OH \\ OH \end{matrix} + PO_4^{---} + 3H^+ \qquad (2)$$

Die hierbei entstehenden Wasserstoffionen bewirken, daß der pH-Wert erniedrigt wird (Tabelle 1, b, c). Weiterhin reagieren sie gleichgewichtsmäßig mit den in Lösung gegangenen PO_4^{---}-Ionen: $PO_4^{---} + 3H^+ \rightleftharpoons HPO_4^{--} + 2H^+ \rightleftharpoons H_2PO_4^- + H^+ \rightleftharpoons H_3PO_4$. (3) Ein Gleichgewicht ist erreicht, wenn die (aktuelle) Wasserstoffionenkonzentration und die PO_4^{---}-Ionenkonzentration dem Austauschgleichgewicht entsprechen. – Bei der Umsetzung von Aluminium- und Eisen-Hydroxydphosphat mit Natriumacetat–Essigsäure-Pufferlösung (pH = 7) werden ebenfalls OH^--Ionen gegen PO_4^{---}-Ionen unter Freiwerden von Wasserstoffionen ausgetauscht (Erniedrigung des pH: Tabelle 1, b, c). Letztere werden aber zum größten Teil mit den von der Pufferlösung gelieferten Hydroxylionen zu Wasser verbunden, sodaß die Gleichung (2) weiter von links nach rechts verlaufen kann, und große Mengen Hydroxylionen gegen Phosphationen ausgetauscht werden, bis ein Gleichgewicht erreicht ist. In gleicher Weise werden bei der Berührung von Aluminium- und Eisen-Hydroxydphosphat mit Glykokoll-Lösung die frei werdenden Wasserstoffionen zum größten Teil abgepuffert, sodaß die Umsetzungen nach Gleichung 2 und 3 weiter von links nach rechts verlaufen können und viel Phosphat sich im Gleichgewicht in Lösung befindet (Tabelle 2, b, c).

c. Umsetzung von Tonmineralen mit Wasser und Glykokoll-Lösung.

Bei Einwirkung von reinem Wasser auf Tonminerale, die z.T. mit Kaliumionen belegt sind, findet ein Austausch von Wasserstoffionen (aus dem Wasser) gegen Kaliumionen (aus dem Tonmineral) statt:

$$KR + HOH \rightleftharpoons HR + KOH. \qquad (4)$$

Schon bei der Entstehung einer geringen Kaliumkonzentration entsteht eine so hohe Hydroxylionenkonzentration (Tabelle 3), daß

das Austauschgleichgewicht erreicht ist. Bei der Einwirkung von Glykokoll-Lösung auf den gleichen Ton werden die entstehenden Hydroxylionen zum größten Teil mit Wasserstoffionen zu Wasser verbunden, und die Umsetzung nach Gleichung (4) kann so weit von links nach rechts verlaufen, bis eine (aktuelle) Hydroxylionenkonzentration und eine Kaliumkonzentration entstanden sind, die dem Austauschgleichgewicht entsprechen. – Bei Berührung von Ton mit Natriumacetat-Essigsäure-Pufferlösung treten die gleichen Umsetzungen auf. Wegen des Austausches von Natriumionen (aus der Pufferlösung) gegen Kaliumionen (aus dem Ton) ist der Einfluß der potentiellen Wasserstoffionenkonzentration allein jedoch nicht zu erkennen.

d. Zur allgemeinen Erscheinung der Einwirkung von Pufferlösungen auf Mineralien.

Die diskutierten Umsetzungen dürften in der zur Verfügung stehenden Literatur noch nicht beschrieben worden sein. Die allgemeine Erscheinung der Einwirkung von Pufferlösungen auf Salze von starken Basen und schwächeren Säuren sowie auf Kationen- und Anionenaustauschersubstanzen ist jedoch öfter theoretisch behandelt worden (z.B. [1] [2]). Daher werden mit steigender potentieller Wasserstoffionenkonzentration nicht nur die Phosphat- und Kaliumkonzentrationen im Löslichkeits- bzw. Austauschgleichgewicht der Phosphat- und Kaliumminerale erhöht, sondern allgemein werden durch die Erhöhung der potentiellen Wasserstoffionenkonzentration die Kationen- und Anionenkonzentrationen im Gleichgewicht mit nahezu allen Mineralen (Ausnahmen: Salze starker Säuren, z.B. Sulfate) vergrößert. Diesbezügliche Untersuchungen könnten weitere für die Pflanzenernährung und Bodenbildung wichtige Erscheinungen aufzeigen und erklären.

2. *Beispiele für den Einfluß der potentiellen Wasserstoffionenkonzentration auf die Phosphat- und Kaliumkonzentration im Gleichgewicht einiger Phosphat- und Kaliumionen enthaltender Substanzen*

Die Erhöhung des Löslichkeitsgleichgewichtes von Hydroxylfluorapatit und der Austauschgleichgewichte der koexistenten Aluminium- und Eisen-Hydroxydphosphate mit steigender potentieller Wasserstoffionenkonzentration dürfte die Erscheinung er-

klären, warum in der Bodenlösung bedeutend höhere Phosphatkonzentrationen vorhanden sind als im Löslichkeitsgleichgewicht des Hydroxylfluorapatits. So sind z.B. in Böden mit gutem Kulturzustand, die eine intensive bakterielle Tätigkeit aufweisen und somit viel gelöste Kohlensäure bei annähernd neutraler Reaktion enthalten, bis 50 mg P_2O_5/l in der Bodenlösung vorhanden [3], während im Gleichgewicht des Hydroxylfluorapatits und der koexistenten Aluminium- und Eisen-Hydroxydphosphate nur 0,2 mg P_2O_5/l vorkommen. Auch die bekannte landwirtschaftliche Beobachtung, daß durch verbesserte Bodenbearbeitung allein eine verstärkte Lieferung von Phosphorsäure und Kali an die Pflanzen erfolgt, dürfte sich durch die erhöhte bakterielle Tätigkeit und somit höhere Kohlensäurekonzentration und höhere potentielle Wasserstoffionenkonzentration des Bodens erklären lassen. Die hohe Löslichkeit von Calciumphosphat bei hoher potentieller Wasserstoffionenkonzentration dürfte weiterhin die Ursache sein, daß eine Bestimmung der "Löslichkeit" von Phosphorsäuredüngern mit ammoniakalischer Citratlösung (Petermannscher Lösung) trotz deren alkalischer Reaktion (geringe aktuelle Wasserstoffionenkonzentration) möglich ist.

ZUSAMMENFASSUNG

Es wurde gefunden, daß die Phosphat- und Kaliumkonzentrationen im Löslichkeits- bzw. Austauschgleichgewicht der Phosphorsäure- und Kaliummineralien des Bodens (Hydroxylfluorapatit, Aluminium- und Eisen-Hydroxydphosphat, Ton) mit steigender potentieller Wasserstoffionenkonzentration erhöht werden. Diese Erscheinung könnte einige landwirtschaftliche und bodenkundliche Befunde erklären.

Eingegangen am 21. November 1960

LITERATURVERZEICHNIS

1 Hägg, G., Die theoretischen Grundlagen der analytischen Chemie, Basel 1950, S. 58 ff.
2 Nernst, W., Theoretische Chemie, Stuttgart 1926, 11. bis 15. Aufl. S., 587 ff.
3 Rathje, W., Zur Kenntnis der Phosphate VI: Das Phosphatgleichgewicht im Boden. Bodenk. u. Pflanzenernähr. **38**, 129 (1942).
4 Rathje, W., Die Anionenaustauschersubstanzen Aluminium- und Eisen-Hydroxyd-Silikat-Phosphat-Humat als Minerale des Bodens, Plant and Soil **13**, 75 (1960).
5 Tischer, J., Über die Bestimmung der Phosphorsäure mittels der Molybdänblau-Methode, Z. Pflanzenernähr. Düng. u. Bodenk. **33**, 192 (1933).